P. V. Mohanan *Editor*

Microfluidics and Multi Organs on Chip

Springer

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Historical and Technological Background to 'Organ on a Chip'

1

J. Malcolm Wilkinson

Abstract

This chapter covers the history, technology and market requirements that are driving the development of organ on a chip (OOAC), also known as microphysiological systems (MPS). It covers the challenges of translating a new technology from research to the marketplace. It opens with an analysis of the origins of the technology from the microelectronics industry which then extended into new materials other than silicon and diverged into microelectromechanical systems. Commercial successes using microfluidics are described and the struggle to build more complex lab-on-a-chip devices and ultimately the application of these technologies to biological systems. Although this started over 20 years ago, the development has been hampered by a series of new technical problems which are elaborated. The chapter closes with an analysis of the remaining challenges and a road map of how some current testing using animals could be replaced. The conclusion is that a new realism has entered the market forecasts for OOAC which is now forecast to reach $303 M by 2026. The author's opinion is that because of the demand for a replacement for animal testing, the market could be even larger and reach $529 M by 2027. Meanwhile the technological developments and research continue.

Keywords

NAMS—non-animal methods · OOAC—organ on a chip · MPS—microphysiological systems · Microfluidics · Market forecasts

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1.1 Introduction

This chapter covers the history, technology and market requirements that are driving the development of 'organ on a chip' (OOAC), also known as microphysiological systems (MPS). It pays particular attention to the challenges of translating a new technology from research to the marketplace. It opens with an analysis of the origins of the technology from the semiconductor microelectronics industry and then follows the extension of the methods into new materials other than silicon and the divergence into MEMS, micro-electromechanical systems. The commercial successes using microfluidics are then described as well as the struggle to build more complex lab-on-a-chip devices. The final part of the story is the application of these technologies to biological systems. This started over 20 years ago, but the development has been hampered by a series of new technical problems. The chapter closes with an analysis of the remaining challenges and a roadmap of how the market could develop to replace some of the applications that are currently served by using animal testing.

1.2 Origins of Microchip Technology

The use of the term 'organ on a chip' suggests a link to the semiconductor electronic chip and it is true that the technology has evolved from the first silicon chips developed in the 1960s. However, there are significant differences between electronic chips and biological chips. Electronic chips are built from semiconductor materials and are designed to store and manipulate electronic charges. Biological chips are built from a wide range of plastic and glass materials and are designed to handle fluids and living cells. The dimensions of cells are thousands of times larger than the latest transistor electronic circuits.

The progress in microchips from the first devices that contained as few as 10 transistors to the current generation of computer chips that have over 100 million transistors has been truly amazing. The underpinning manufacturing technology (photolithography) has continually shrunk the dimensions of the transistors from 10 μm in the 1970s (1 μm = 1 millionth of a metre) down to 1 μm by the year 2000. Today, new electron beam technologies have replaced optical printing to achieve dimensions of a few nanometres (1 nm = a thousandth of 1 μm).

The incredible technical progress has been driven by commercial benefits that come from the improved performance of smaller transistors—they are faster, consume less power and are cheaper (per transistor). The progress has been encapsulated in the so-called 'Moore's law' [1]. This has successfully modelled the shrinking of the transistor dimensions and the resultant doubling of chip complexity every 3 years. The progress in electronics has led to an emerging dogma that 'smaller means better' as we have progressed from milliscale to microscale and now nanoscale. However, this dogma must be questioned when we are dealing with devices that are not electronic but are trying to control liquids or biological materials.

The behaviours of biological fluids and cells are governed by different physical laws than electrons. Electrons move around electronic chips under the influence of electric fields whereas biological fluids and cells move under the influence of hydrostatic pressure and concentration gradients. Physical effects such as viscosity and surface tension can be dominant in biological devices whereas they are of no relevance to electronic circuits.

1.3 Second Phase of Technology: New Materials and Applications

By the 1990s, it was found that photolithography could be applied to many other materials, not just silicon chips. Metals, glass, ceramics and some plastic materials could also be shaped. Where chemical etching could not be used, the use of plasma or lasers could extend the types of structures that could be made. 'Chips' now come in many flavours!

The devices were also combining different physical effects, not just electronic, but mechanical, thermal and piezoelectric. The second wave of technology became known as MEMS (micro-electromechanical systems) or just 'microsystems'. There was an explosion of new technology, but companies were searching for 'killer applications' to match the commercial success of electronics. By the end of the millennium, several had been found including inkjet print heads, automotive pressure sensors and accelerometers to name just three [2]. The dogma of 'smaller is better' still seemed to apply but inkjet printing showed that there could be an optimum size—which in this case was the resolution of the optical printed image. The amazing commercial success of inkjet was based on an interesting business model: the printing machines were sold at very low profit margins as the profits came from the sale of the proprietary inkjet cartridges. Although the first cartridges were single use, a business has now developed to recycle and refill cartridges.

The success of these first fluid devices triggered the interest of many chemists to investigate the potential of the microsystems chip technology for chemical analysis and synthesis. The goal was to create a chemical 'lab on a chip'. However, the technical challenge of how to move fluids into and around these chips had still to be solved.

1.4 Third Phase: Microfluidics and Micropumps

Inkjet printing had established that liquid flow can be created inside milliscale or even microscale devices. The major technical innovation was not so much producing the capillary channels, but the design of transducers to pump the fluids. Several competing technologies were successfully developed. Hewlett-Packard used thermal heating of the liquid ink on the chip to vaporise it. This generated gas pressure that used to eject a droplet of ink. The piezoelectric effect, whereby electric voltage changes the shape of a crystal, was an alternative way to squeeze a channel

containing ink. As the liquids were incompressible (unlike a gas), this would eject the ink from the channel through a nozzle as a jet onto the paper.

Many companies were developing micropumps and searching for alternative applications other than printing. Several different technologies were tried in the late 1990s but most had the disadvantage that they could not sustain flow when there was backpressure. Backpressure can come from resistance to fluid flow in a long narrow channel or might be caused by a blockage. (Blockages are particularly troublesome for biological fluids.) For laboratory-on-a-chip applications, it was thought that it would be advantageous to try to build pumps on the same chip as the microfluidic channels. A 'lab on a chip' would ideally be able to mix reagents, control chemical reactions and use on chip sensing to analyse the results. Very few of these concepts worked reliably, often hampered by apparently simple problems like air bubbles blocking the flow. This is because at the microscale, capillary forces, viscosity and surface tension dominate the flow. Some devices incorporated bubble traps, but most were not effective or took up more area on the chip than the actual functional device. This is a problem because the manufacturing cost is critically dependent on the area of the device very few of the pump designs proved reliable, but some have been qualified for medical use and are in volume production [3].

Although surface tension capillary forces can be a problem, they can also be very useful to move low-viscosity fluids around. Low-cost manufacturing processes can be used to create sandwich structures so that samples of blood, urine or other biological fluids are pulled through the structure by surface tension. The market for these so-called lateral-flow diagnostic kits has grown considerably with applications such as pregnancy testing and more recently coronavirus disease (COVID) testing. Even blood samples (blood is a viscous non-Newtonian fluid) can be moved in a capillary channel when combined with a suitable primer solution to lower the viscosity. This is the method used in the iSTAT blood testing cartridge [4]. These widely used lateral-flow devices do not need a micropump!

1.5 Fourth Phase: Moving into Cell Culture

With the growing interest in microfluidics and 'lab on a chip' in the 1990s, several biologists studying in vitro cell culture started to look at the potential for culturing living cells on a chip. Professor Michael Shuler at Cornell was one of the pioneers [5]. He filed patents and formed a start-up company, Hurel Corporation. Although his vision was to connect multiple chambers containing different types of cells, he started with a simpler structure and focused on liver models for toxicity testing. It became clear that introducing biological materials into microfluidics created some complex problems and there were many technical challenges to solve. One of these was that liver cells turned out to be difficult to keep alive for more than a few days as they are very sensitive to flow stress. Microfluidic structures can easily produce high flow stress, even at low flow rates. It is necessary to have enough flow to bring oxygen and nutrients to the cells (to support metabolism) without causing them to differentiate because of the high flow stress.

Shuler was keen to minimise the size and scale of his chip designs because his goal was to build chips with large numbers of chambers to facilitate high throughput screening (HTS) useful for toxicity screening in the pharmaceutical industry. The pharmaceutical industry has libraries of hundreds of thousands of compounds produced by combinatorial chemistry [6]. The objective is to quickly screen large numbers of compounds to select those that might have the required biological activity on a target molecule. This requirement had driven the microtitre plate testing market towards 96 well or even 384 well plates [7]. These devices have large numbers of unconnected open wells with no requirement to flow cells down the capillary channels. Hence, they sidestep the problems of flow stress, bubbles and blockages.

Another Professor, Arti Ahluwalia in Pisa University, took a different approach. Her goal was to find a replacement for the use of animals in drug testing and she developed the principle of allometric scaling for organ-on-a-chip (OOAC) devices [8]. This principle suggested that one of the most important factors in creating viable multi-organ models is the quantity ratio of the different cell types. She also recognised the importance of flow stress as a factor causing cells under in vitro conditions to differentiate away from the required phenotype. Her team in Pisa also established ways to optimise flow through chambers to support the viability of sensitive cells like hepatocytes or neurons [9]. It was recognised by these early pioneers of 'organ on a chip', that flow of media, three-dimensional (3D) structures (tissue-like) and multiple cell types were essential to create physiologically relevant models. Physiologically relevant models are required for accurate prediction of chemical toxicity, drug efficacy and models of human disease. A further improvement is expected from the use of human cells rather than animal cells.

It is typical that in the early stages of any new technology development cycle there are few standards. Even today, over 15 years after the early developments of 'organ on a chip', there are no standards and a great diversity of chip sizes and fabrication materials. This variety hampers the adoption of the technology by the mainstream market of biologists or toxicologists, who would prefer simple easy-to-use technologies. In contrast, most current users of OOAC are pioneers and early adopters who are prepared to work on the teething problems. Nearly all early OOAC devices require a complex 'life support' system round the chip to achieve viable cultures over more than a few hours. Temperature and gas control are fairly straightforward using standard laboratory incubators that run at 37 °C with a partial oxygen pressure of 20% and 5% CO_2. However, there is little agreement about the best way to provide flow of media over the cells.

Syringe pumps, peristaltic pumps and gravity-fed systems are all still in use. A research group in Massachusetts Institute of Technology (MIT), Boston, USA, believed that it would be advantageous to incorporate pumping devices on the OOAC device itself. A large number of research groups followed the MIT lead and tried to build pneumatic pumps using piezoelectric materials [10, 11]. These require high voltages to be applied to the chip, which are not particularly compatible with moist biological devices. The addition of piezo materials to the chip-making process also increases its complexity and so severely compromises the cost and

yield. To avoid these issues, several groups leave the pump valves on the chip but have moved the pumping pressure generation to an externally switched system utilising bottled gas and vacuum supplies, which are commonplace in many large labs. TissUse GmbH in Germany and CNBio in the UK use this method [12, 13].

Some recent organ-on-a-chip developments are moving away from on-chip pumps to minimise the complexity of the chip design and fabrication. This can simplify the operating protocols for biologists who are not familiar with microfluidics, pressure and flow [14]. This recognition that ease-of-use is a key factor for the adoption of new technology has come very late to many of the companies developing organ-on-a-chip devices.

1.6 Fifth Phase: Translation to the Market

Translation to the market is not so much about continuing technical development but is more about building a market and a business to serve that market. This section focuses on the commercial challenges and how they might be overcome. Currently, most of the groups developing OOAC are either academic research teams or start-up companies coming out of academia. Such organisations face many problems, irrespective of the technical challenges:

- Their products tend to be a result of *technology push* rather than *market pull* and hence do not necessarily focus on solving customers' needs.
- They have *insufficient funding* from seed-corn funds and do not have the resources to finance the long development process to get a product to the market [15].
- Their team is heavy on technical skills but light on commercial and market experience.

The various business models being explored by start-up companies are:

- Product sales
- Contract research services
- Collaborative research funded by grants
- Technology licensing

Any of these approaches can sustain a business but demand different strategies, levels of initial finance and a different profile of technical and commercial skills in the company.

Successful high-tech firms tend to follow the guidelines first presented in the book *Crossing the Chasm* by Geoffrey Moore [16]. Moore made the point that there is a significant difference between the requirements of early adopters of a new technology and those users in the mainstream market. This can be characterised as a 'chasm' and is illustrated in Fig. 1.1. Moore recommended that companies should aim to get the product out into the hands of the customers as soon as possible. This

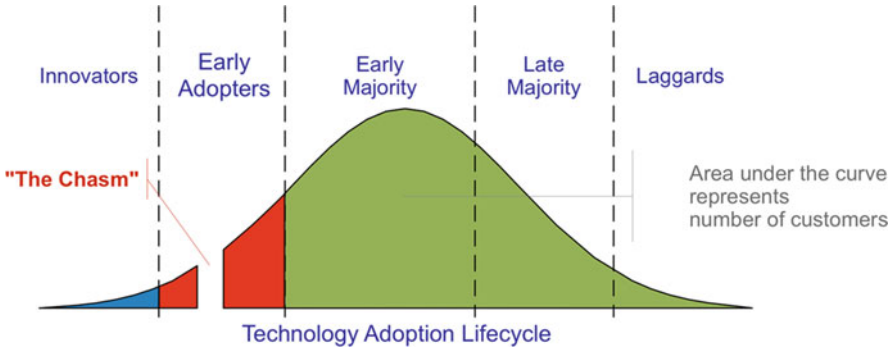

Fig. 1.1 The gap or chasm in the early market for new technologies. The market develops on a timeline running from left to right. The area under the curve represents the cumulative number of customers

not only generates early revenue but also gives the company customer feedback that it should use to refine the product specification and implementation. One of the 'organ on a chip' companies that has tried to follow this mantra is Kirkstall Ltd. [17]. The company was founded in 2006, released its first product in 2009 and second-generation product in 2016. It is set to release its third-generation product in 2021, incorporating the experiences and feedback from over 70 customers who have used its earlier products.

Embryonic companies have to build a sustainable business model that will see them through the 'valley of death' that faces most start-ups when their initial funding (from grants or venture capital) dries up. New companies are busy solving technical challenges, building a new management team and trying to create a market for their products, all at the same time. There is usually a misconception that the market is already there, and customers will beat a path to their door if the product is good enough. Any successful business depends on a sound understanding of the market needs and the dynamic and competitive forces in that market, and these have been well described by Michael Porter [18].

New markets are difficult to forecast. Early forecasts predicted that the OOAC market would be several $billions by 2025 [19] but a recent reassessment by the same market research analysts has suggested that the combined revenue of the top ten companies was only $20 million in 2020. A further challenge is when a new market is developing and the requirements of the different sectors of that market are conflicting. This is certainly true for 'organ on a chip' where there are two major commercial opportunities: firstly, replacing the use of animals in academic medical research, and secondly, transforming the drug development pipeline in the pharmaceutical industry. There are other opportunities in the cosmetic and chemical safety testing that are closely related to the pharmaceutical industry requirements. The different opportunities in the pharmaceutical sector are illustrated in Fig. 1.2. Further analysis of how the different sectors of the market might develop is given in the next section.

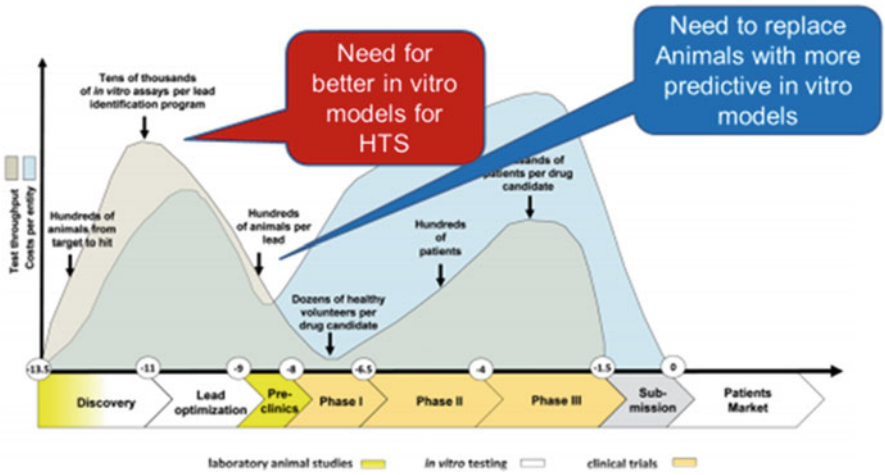

Fig. 1.2 The drug discovery and development timeline. This highlights the different phases at which in vitro and animal testing are employed

1.7 Status Report and Remaining Challenges

One of the earliest status reports and competitive analysis of commercial activity in OOAC was presented by the current author at a conference in Amsterdam in 2016 [20]. This highlighted the differences between the new start-up companies in terms of funding, technology base and engagement with customers. Table 1.1 illustrates how rapidly the OOAC market has been evolving since that report.

Another source of information on the status of the technology is an excellent meta-analysis and translational roadmap presented by Allwardt et al. [21]. This not only maps the current status of worldwide efforts on OOAC but also points out the areas where improvement is needed for commercial viability. These include technological maturity, more robust validation of translational and predictive in-vivo-like biology, and requirements of tighter quality standards. The paper shows a linear roadmap to commercial adoption that begins with device development, followed by application development and then enters the translational stage that enables specific regulations and standardisation, and provides a level of ease-of-use.

OOAC is still not a mature technology, and the applications and business models are still in a state of flux. Here are some of the challenges remaining before the technology can achieve its widely trumpeted potential:

- Better understanding of the market/application needs.
- Simplification of the technology.
- Development of assays and standard protocols based on the OOAC technology.
- Technical validation of better prediction from the physiological models.
- Proof of benefit to justify users shifting to new methods.
- Engagement with the regulatory bodies to validate and accept new methods.

Table 1.1 Historical data on the development of OOAC from 2007 to 2020

Measure	2007	2012	2017	2020
Government grants/period	$10 M over 3 years	$200 M over 5 years	$22 M over 10 years	$35.5 M over 5 years
Venture capital investment (examples)		$28 M (emulate + Tara + Hurel)	$57 M (emulate + Mimetas)	$82 M (emulate)
Number of start-up companies (cumulative)	3	8	18	40
Number of OOAC publications	5770	11,100	14,400	17,800
Market size	<$2 M	~$4 M	~$8 M	~$20 M
Number of different organoids connected	1	3	3	5
Organoid types	Liver Skin	+ Lung + Bone + Gut +++	+ Heart + Brain +++++ (16)	

The multiple plus signs give an indication of the number of additional organs that have been modelled
Source: Author's own research

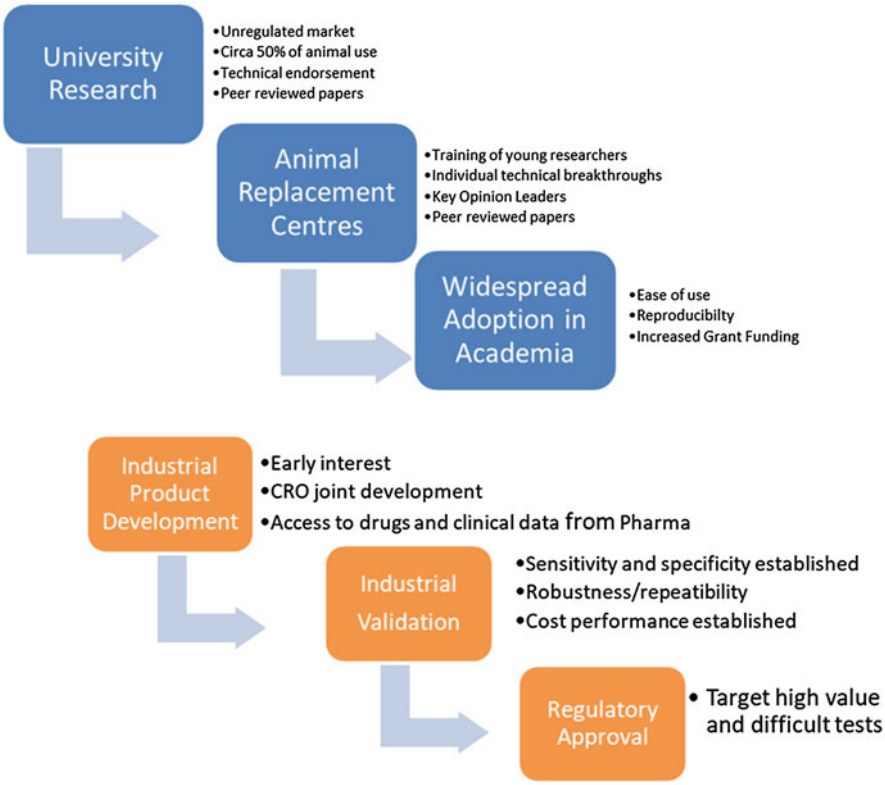

Fig. 1.3 A roadmap and strategy for accelerating the adoption of alternative methods. The adoption by industry and academia of OOAC technology will proceed in parallel. It is the author's view that the industry take-up will lag because of the factors identified in Sect. 1.8 of this chapter

• Development of necessary skill sets in the research community so they can handle the increased complexity of multi-organ models.

1.8 Roadmap and Conclusions

This author believes that OOAC adoption by academic researchers and commercial users will proceed in parallel, and this is shown in Fig. 1.3. The rationale is that the market requirements for replacement of animals in academic medical research are quite different from those of the pharmaceutical and chemical companies that need improved in vitro methods with high throughput for toxicity and efficacy testing.

The following paragraphs list the factors driving and resisting change in both industry and academia:

Drivers for change in the pharma and industry:

- Drug development pipeline is failing to deliver new drugs.
- Animal testing fails to identify side effects of new drugs.

- Human stem cells becoming more available.
- 3D and perfusion models are more physiologically relevant.
- Regulatory changes in cosmetics industry restricting use of animals.

Barriers to adoption of new technology in pharma and industry:

- Validation of economic benefits needed.
- Inertia—capital investment in existing methods.
- Regulatory changes needed for adoption of animal alternatives.

Drivers for change in academic research:

- Ethical arguments against the use of animals.
- Scientific arguments for use of human cells.
- In vitro is cheaper than use of animals.
- Change to non-animal methods does not require regulatory approval.

Barriers to change in academic research:

- Shortage of research funding.
- Inertia—existing career paths based on use of animal models.

The conclusion is that the adoption of new non-animal methods will be much faster in academic research than in the regulated pharmaceutical industry. This conclusion is supported by the way momentum for change in academia is rapidly building:

- The European Union is driving regulatory change and increasing grant funding for animal replacement (AR) research.
- Two leading UK universities have set up animal replacement centres (ARCs).
- Canada and Italy both have created labs dedicated to AR.
- The Netherlands and Sweden have set timescales for phase out of animal methods.
- *ALTEX Journal* dedicated to AR methods.
- Adoption of new technology by industry will follow.
- Evidence from academic research will support regulatory change.
- Academics can 'debug' the new technology.
- Validation of economic benefits can follow academic studies.

1.9 Conclusion

In conclusion, it appears that a new realism has entered the market forecasts for OOAC. A recently published report [22] has suggested that by 2026 the market will be $303 M with a 39.9% cumulative annual growth rate (CAGR). The author's

opinion is that the replacement of animal testing could be even larger, with the market for OOAC or microphysiological systems reaching $529 M by 2027 [23]. Meanwhile, the technological developments and research would continue. Those readers who require more technical information are advised to study the review article by Wu et al. [24].

References

1. Moore's Law. https://www.intel.co.uk/content/www/uk/en/silicon-innovations/moores-law-technology.html
2. https://www.edn.com/report-highlights-emerging-killer-apps-for-mems/
3. https://www.debiotech.com/jewelpump/
4. https://www.pointofcare.abbott/int/en/offerings/istat/istat-handheld
5. Camp JP, Stokol T, Shuler ML (2008) Fabrication of a multiple-diameter branched network of microvascular channels with semi-circular cross-sections using xenon difluoride etching. Biomed Microdevices 10(2):179–186. https://doi.org/10.1007/s10544-007-9123-x
6. Liu R et al (2017) Combinatorial chemistry in drug discovery. Curr Opin Chem Biol 38:117–126. https://doi.org/10.1016/j.cbpa.2017.03.017
7. Manns R (1999) Microplate history. In: Presented at MipTec-ICAR'99, May 17–21, 1999, Montreux, Switzerland, 2nd edn
8. Ucciferri N, Sbrana T, Ahluwalia A (2014) Allometric scaling and cell ratios in multi-organ in vitro models of human metabolism. Front Bioeng Biotechnol. https://doi.org/10.3389/fbioe.2014.00074
9. Mazzei D, Guzzardi MA, Giusti S, Ahluwalia A (2010) A low shear stress modular bioreactor for connected cell culture under high flow rates. Biotechnol Bioeng 106(1):127–137. https://doi.org/10.1002/bit.22671
10. Griffiths L (2003) Liverchip technology. MIT Technology Review
11. Xiao S, Coppeta J, Rogers H et al (2017) A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle. Nat Commun 8:14584. https://doi.org/10.1038/ncomms14584
12. TissUse GmbH reference. https://www.tissuse.com/en/technology/
13. Tsamandouras N, Chen WLK, Edington CD, Stokes CL, Griffith LG, Cirit M (2017) Integrated gut and liver microphysiological systems for quantitative in vitro pharmacokinetic studies. AAPS J 19:1499. https://doi.org/10.1208/s12248-017-0122-4
14. Brown J (2021) Keynote paper at ACTC conference, Vanderbilt University, 30 Jun 2021
15. Fitzgerald AM, White CD, Chung CC (2021) Stages of MEMS product development. In: MEMS product development. Microsystems and nanosystems. Springer, Cham. https://doi.org/10.1007/978-3-030-61709-7_3
16. Moore G (1991) Crossing the chasm. Harper Collins, New York. ISBN 0-06-051712-3
17. https://www.kirkstall.com
18. Porter ME (1980) The competitive strategy: techniques for analyzing industries and competitors. Free Press, New York. ISBN-13 978-0743260886
19. Clerk S, Villien M (2017) Organs-on-chips 2017. In: Market & technology report available from Yole Developpement, Le Quartz, 75 Cours Emile Zola, 69100 Lyon-Villeurbanne, France
20. Wilkinson presentation. https://www.micronanoconference.org/app/uploads/sites/57/2016/12/Organ-on-a-Chip_The-Commercialisation-Report-Card-2016_Malcolm-Wilkinson_Kirkstall.pdf

21. Allwardt V, Ainscough AJ, Viswanathan P, Sherrod SD, McLean JA, Haddrick M, Pensabene V (2020) (Review article) Translational roadmap for the organs-on-a-chip industry toward broad adoption. Bioengineering (Basel) 7(3):112
22. https://reports.valuates.com/market-reports/QYRE-Auto-26C1860/global-organ-on-chip
23. MANCEF COMSWORLD (2021) Virtual conference, 16–17 Sept 2021. https://www.mancef.org/china-comet-2021/
24. Wu Q, Liu J, Wang X et al (2020) Organ-on-a-chip: recent breakthroughs and future prospects. BioMed Eng Online 19:9. https://doi.org/10.1186/s12938-020-0752-0

Applications of Microfluidics

2

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Abstract

Microfluidics as a field has a plethora of applications in several fields. From heat transfer to biomedical applications, microfluidic techniques are used to deliver solutions. In the present chapter, we look into the basics of microfluidic techniques used to manipulate tiny volumes of fluids. Further, a detailed discussion on acoustofluidics, lab/organ-on-chip, biosensing, and cell manipulation follows. Section 2.4 focuses on the use of bulk and surface acoustic waves to manipulate particles and cells. Section 2.5 sheds light on the use of microfluidic chips mimicking an organ or its basic process and how the same is used to study the effect of drugs on the organs. Section 2.6 focuses on using microfluidic techniques for disease detection and prognosis monitoring. The part on Cell manipulation cuts through various active and passive techniques for cell trapping, focusing, and sorting.

Keywords

Microfluidics · In-channel · Open-surface · Acoustofluidics · Lab-on-chip · Biosensing · Cell manipulation

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2.1 Introduction

The field of microfluidics deals with scales ranging from millimeter to micrometer to manipulate fluids and particles. The higher surface area to volume ratio at such tiny length scales enables a range of applications to solve challenging problems. In terms of the operating platform, microfluidics can be classified into open-surface and in-channel platforms. In open-surface microfluidics, the surface is open to the ambient condition and the droplet is manipulated using surface morphology and wettability characteristics. Similarly, in-channel microfluidic techniques are those where the droplet is manipulated inside a closed channel. Again, in terms of operating techniques, microfluidics is classified into active and passive techniques. Active techniques include acoustic [1], electric [2], and magnetic [3] to manipulate fluid, whereas in passive techniques surface wettability [4], non-inertial lift [5], etc. are used to do the same. This chapter includes a detailed discussion on basic techniques used in microfluidics and their applications. The differences and advantages of in-channel and open-surface microfluidics have been explored in the first two sections. In the subsequent sections, acoustofluidics, lab/organ-on-chip, biosensing, and cell manipulation applications have been explored and discussed in detail.

2.2 In-Channel Techniques

The in-channel-based microfluidics can be broadly classified into continuous-flow microfluidics and droplet-based microfluidics. Continuous-flow microfluidics deals with the manipulation of particles or cells in fabricated microdevices without any breakup of liquid. A few major handling tasks in continuous-flow microfluidics are sorting, separating, and trapping microparticles or cells; mixing reagents, liquids, etc. Various applications of the in-channel techniques are discussed in this section.

2.2.1 Continuous-Flow-Based In-Channel Microfluidics

The continuous-flow-based in-channel microfluidics can be further divided into passive and active methods. In passive methods, the fluid-flow characteristics and device design are primarily used to guide the particles or cells inside the microchannel without any external stimulus. On the other hand, active methods use external energy, which in turn influences the particle or cell motion, fluid mixing, etc. The passive techniques are advantageous over active techniques since no external energy sources are required for actuation, thus reducing the costs of the system. In the case of active techniques, the efficiency of sorting of particles and mixing of reagent is higher; therefore, high throughput can be achieved. Further, combined techniques are also developed, which uses external energy sources in a passive microfluidic device to enhance efficiency.

In the last decades, several review papers have discussed the state-of-the-art microfluidics sorting and separation techniques. For example, a comprehensive review on various microfluidics technique used for separation and sorting of micron-sized neutral particles were discussed by Sajeesh and Sen [6]. An extensive study of the manipulation of cells and particles suspended in a viscoelastic medium is presented by Zhou and Papautsky [7]. The applications of microfluidic techniques for biological applications, viz., label-free isolation of cells, quantitative biology, detection and separation of cancer cells, cell affinity, detection of DNA (Deoxyribonucleic Acid), etc., are discussed extensively in the literature [8–12]. We briefly discuss the different active and passive sorting and separation techniques in the following section.

2.2.2 Sorting and Separation

Various passive and active methods have been exploited to achieve micro-objects' sorting and separation in a continuous-flow microfluidics device. Passive methods can be classified as pinched-flow fractionation (PFF), inertial and Dean flow, microvortex manipulation, deterministic lateral displacement (DLD), and filtration. We will briefly discuss each of the methods below. In PFF, continuous sizing of microparticles can be achieved using the various characteristics of laminar fluid flow [13]. The particle fluid mixture is focused using a particle-free fluid, resulting in a "pinched segment" inside the microchannel. The fluid particles' mixture coming out of the "pinched segment" can be separated depending on the particles' sizes [14]. The technique can be used for any type of particle as particle concentration does not affect the separation efficiency. Further, by adjusting the dimension of the outlet channels, the displacement of the particles can be controlled. Such asymmetric pinched-flow fractionation (asPFF) is used to separate spermatozoa from erythrocytes depending on the tumbling motion of spermatozoa [15].

A particle experiences two inertial lift forces while moving along a straight microchannel: (1) shear-gradient-induced lift force, and (2) wall-effect-induced lift force [16]. The parabolic nature of the fully developed velocity profile gives rise to the shear-gradient-induced lift force, which directs the particle to move toward the wall. On the other hand, when a particle is near the wall, the flow around it gets disturbed, resulting in the formation of asymmetric wake around the particle. The asymmetric wake leads to the wall-effect-induced lift force that pushes the particle away from the microchannel wall [17]. Superimposed effects of these two forces lead to lateral particle migrations, and the positioning of the particle will be different depending upon the sizes of the microparticles. Apart from this, deformable particles or cells can be sorted using the deformation-induced drag force, which pushes the object to the center of the microchannel [18].

In the case of the curved microchannel, the secondary flow or Dean flow is created due to centrifugal effects, which can be utilized to manipulate the equilibrium position of the particles [19]. The secondary or Dean flow induces drag force on the particles, and thereby the particle position can be manipulated due to the

combined effects of shear-induced lift force and the Dean drag force [20]. Here, the particle equilibrium position depends on the Re (Reynold's number) and the ratio between lift force and Dean drag force [21]. The advantage of using a curved microchannel is that it requires less distance to focus the particles than a straight microchannel and thus reduces the hydrodynamic resistance. One popular method used to separate cells based on size and deformability is deterministic lateral displacement (DLD) [22]. An array of obstacles with a size larger than the cells is created in the fluid domain. When a cell moves through this array, it selects the path deterministically based on its size and deformability [23]. Cells with comparable size and deformability traverse equivalent migration length, which leads to higher separation efficiency. The DLD methods are applied to separate particles of different sizes from a mixture [24], and in sorting of non-spherical particles [25], deformable object sorting [26], sorting of circulating tumor cells (CTCs) from normal cells [27], and separation of sleeping parasites from blood [27].

On the other hand, various other active particle sorting and separation methods include dielectrophoresis (DEP), magnetic, optical, and acoustic methods. A neutral particle can get polarized by placing it in a non-uniform electrical field, and the polarized particle experiences a force called dielectrophoresis (DEP) force [28]. DEP has been extensively utilized for electrically controlled trapping, focusing, translation, fractionation, and characterization of particles, chemicals, and biological entities in in-channel microfluidics [29]. In electrophoresis, which utilizes the intrinsic properties of the cells, the process may lead to cell membrane rupture due to Joule heating. In such cases, magnetic techniques are advantageous since they can prevent cell damage and take less sorting time [30]. Further, the microfluidics setup required for such operation is cheaper than other active techniques such as acoustics and optical methods [31]. Magnetic cell separation system (MACS) and its variants, which make use of both magnetic and fluorescent labeling of cells, are widely used since they are more efficient compared to traditional fluorescence-activated cell sorter (FACS) devices [32, 33]. Optical and acoustical techniques utilize the fact that a particle exposed to sound or electromagnetic waves scatters from its surface, resulting in a radiation force that acts on the particle [34, 35]. The radiation force can be used to manipulate the particle equilibrium position inside the microchannel. In the case of the light beam, if the ratio of the refractive index of the particle to that of the medium is less than 1, then the light beam attracts the particle toward the center of the particle due to intensity difference across the interface. By varying the light wavelength and power, the trapping geometry optical methods can be used to sort and separate micron-sized objects. The well-known optical trap method is the optical tweezers that are widely used for deformability measurement and analysis of biological cells [36]. Similarly, when a particle is exposed to a one-dimensional standing wave, it experiences acoustic radiation force (ARF) that arises due to the impedance difference between the particle and the medium [37]. Apart from primary radiation force, particles may experience an interparticle force due to multiple scattering of the acoustic wave from other particles [38], and axial radiation force, which arises due to the spatial variation of the acoustic field [39]. The forces can be exploited to trap particles at the pressure node against the flow, which is beneficial

for washing cells. Various applications of the acoustofluidic techniques are discussed in Sect. 2.4.

2.2.3 Droplet-Based In-Channel Microfluidics

In in-channel-based microfluidics, monodisperse droplets can be generated at high frequencies with picoliter sizes. Usually, droplets are formed as single emulsion created using two immiscible fluids (either water-in-oil or oil-in-water) via passive and active methods [40]. In passive microfluidics, a droplet can be generated by adjusting the volume flow rates of the two immiscible streams in a confinement geometry, which pinches the discrete phase due to interfacial instability [41]. There are mainly three different ways to create droplets using passive methods, viz., T-junction [42], flow-focusing [43], and co-flow [44], as shown in Fig. 2.1a(i), (ii), and (iii), respectively. Further, the formation of double emulsion using two flow-focusing junctions [45] and encapsulation of particles inside a droplet [46] is shown in Fig. 2.1a(iv) and (v), respectively. On the other hand, active droplet generation requires creating a perturbation at the interface through external energy. The various active methods are based on the external energies such as acoustics,

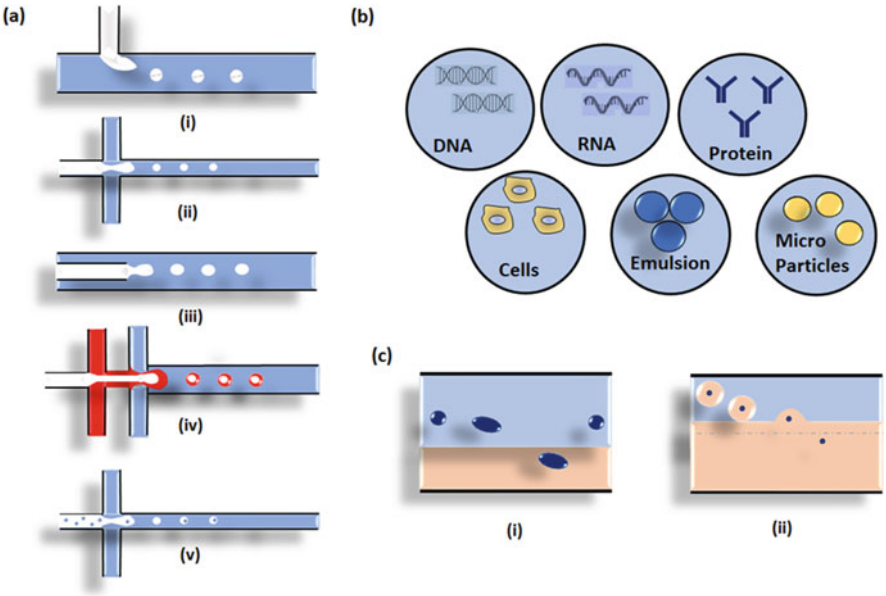

Fig. 2.1 Schematic of (**a**) in-channel droplet generation: (i) T-junction geometry, (ii) flow-focusing geometry, (iii) co-flow geometry, (iv) double emulsion, and (v) particle encapsulation; (**b**) encapsulation of various biological objects, emulsions, and particles; (**c**) (i) transfer of particles across an interface, and (ii) coalescence droplets at a co-flow interface for exchanging microparticles

electric, and magnetic used to initiate the perturbation at the fluid–fluid interface. In-channel-based droplet generation can be manipulated and controlled by the device design, volume flow rates, walls' wettability, fluid properties, the surfactant used, and perturbing variables [47]. The tunability of the above parameters leads to high throughput, which is beneficial to apply for biochemical assaying. Further, droplet microfluidics is advantageous over continuous-flow systems as it brings together scalability and sample isolation. The droplets can be used to encapsulate various biological entities, viz., DNA, RNA, cells, proteins, and emulsions, and microparticles, as shown in Fig. 2.1b. Further, droplet dynamics and coalescence of droplets to a continuous phase in a co-flow arrangement are demonstrated in Fig. 2.1c. Various techniques related to the droplet-based microchannel flows are discussed below.

2.2.3.1 Droplet Fission

Droplet fission is a method to split a droplet or a plug of liquid into smaller droplets, which is helpful in scaling-up the number of droplets. Further, droplet fission can be used to control the droplet concentrations. Both passive and active microfluidic methods can be utilized to achieve droplet fission. Passive methods involve a specific design of the microchannel to control the droplets' sizes without any external stimuli. Traditional polydimethylsiloxane (PDMS)-based microfluidic devices with various channel designs, including T-junctions, obstructions, and branching, are used for droplet fission [48]. Further, splitting a parent droplet into smaller droplets near an immiscible fluid–fluid interface due to shear forces is shown [49]. On the other hand, active methods include using external forces that compete with hydrodynamics, resulting in droplet breakup. Electrowetting-on-dielectric (EWOD) can effectively split a droplet into equal volumes [50]. On-demand droplet fission inside a PDMS microchannel is also demonstrated using surface acoustic waves (SAWs) [51]. A slanted-finger interdigitated transducer (IDT) is used to emanate a surface acoustic wave that exerts an acoustic radiation force (ARF) on the droplets. In-channel splitting of ferrofluid droplet using a T-junction geometry using the intensity of magnetic field has been recently presented [3].

2.2.3.2 Droplet Fusion

Coalescence of two or more droplets is an essential way of mixing and carrying out controlled reactions in an isolated environment [52]. The design of the microchannel plays a crucial role in passive droplets' coalescence. Droplet fusion requires appropriate control of droplets' frequencies, which can be achieved by tuning the flow rates of the continuous and discrete phases and droplet generation design [53]. Further, passive methods include introducing physical obstruction or expansion chambers that facilitate contact between two droplets and induce coalescence [54]. In the case of surfactant-stabilized systems, the surfactant concentrations are altered, which indulge in droplet fusion [55]. Active droplet fusion can be achieved by external forces such as EWOD, acoustics, magnetics, and electric-controlled methods. Different electric-based methods, including EWOD and dielectrophoresis (DEP), have been used to show droplet fusion. Further, droplet fusion has also been

demonstrated using a bulk acoustic wave (BAW) inside a microchannel [1]. Coalescence of droplets with a liquid stream due to acoustic radiation force using a BAW device is also shown [56]. Further, surface acoustic wave (SAW)-based droplet coalescence is also being demonstrated in the literature [57, 58].

2.2.3.3 Droplet Sorting

The droplet microfluidics has an edge over the continuous-flow microfluidics in generating unique droplets that can be examined individually. Sorting of droplets facilitates an enormous number of applications, viz., the encapsulated droplet isolation, purification of synthesized samples, and the segregation of droplets [59, 60]. Further, droplet sorting helps in extracting a single droplet of interest out of its population. Droplet sorting can also be divided into two types: passive and active droplet sorting. Passive methods include the design of the channel geometry, stiffness, and viscosity of the continuous and discrete phases. The channel geometry can be designed such that the continuous flow carries the satellite's droplets. Active droplet sorting can be achieved using magnetic, acoustic, electrical, thermal, and pneumatic control. An excellent review of the various active droplet sorting methods can be found in Xi et al. [61].

2.2.3.4 Encapsulation in Droplets

The droplets can be used to encapsulate biological cells, reagents, and chemicals, which gives rise to a plethora of applications [62]. Encapsulation of particles/cells in confinement, such as droplets, results in an isolated environment for single-cell analysis without contamination. Isolation of a single cell through droplet encapsulation can be achieved primarily by two methods: (1) by focusing and controlling the space between the cells using passive [63, 64] or active techniques [65, 66] so that only one cell arrives at the junction between the discrete and continuous phases at the instant of droplet generation, giving rise to the encapsulation of one cell per droplet, and (2) by perturbing droplet generation due to external forcing (also known as on-demand droplet generation), so that only when a cell arrives at the junction, a droplet encapsulating a single cell is generated. Passive techniques that utilize Dean flow coupled with inertial focusing are used to encapsulate cell inside the droplet. In the case of a curved microchannel, the competition of Dean drag force and inertial lift force subsequently leads to single-cell encapsulation [67]. Encapsulation of a single cell depends on the capillary number and viscosity ratio of the two phases [63]. Further, the frequency of droplet generation can be synchronized to achieve on-demand single-cell encapsulation. A passive technique based on interfacial hydrodynamics is utilized to trap multiple cells, and the release of a cell into a droplet has been exploited [68]. Optical detection and selective isolation of target particles using passive droplet encapsulation have been demonstrated recently by Gaikwad and Sen [69]. A robust technique using an optical method and passive droplet generation technique is developed to inject a precise volume of reagents inside a droplet [65]. The interplay of acoustic radiation force and interfacial tension is being utilized to create droplets from a co-flow system, giving rise to on-demand

droplet generation and encapsulation of particles [70]. Encapsulation of cells inside droplets has also been demonstrated using magnetofluidics [71].

2.3 Open-Surface Techniques

Open-surface microfluidics refers to the manipulation of droplets or other related entities on a surface open to the ambient conditions using active or passive techniques. The ease of modifying substrate wettability and patterning using a range of fabrication methods [72] makes open-surface microfluidics suitable for biosensing and biomedical applications [73]. Simply changing substrate wettability from hydrophilic to hydrophobic gives substantive control over droplet morphology [72]; adding to that the active techniques like acoustofluidics [74] and digital microfluidics [73] enhances the capabilities of open-surface microfluidics even more. Given wetting being the most important factor in open surfaces, it is imperative to understand the governing physics. The wettability of a surface is characterized using the droplet contact angle "θ" on a substrate assuming a chemically homogenous, rigid, and perfectly flat surface, first given by Young's contact angle law $\cos\theta = \frac{\gamma_{sv}-\gamma_{sl}}{\gamma_{lv}}$, where γ_{sv}, γ_{sl}, and γ_{lv} are solid–vapor, solid–liquid, and liquid–vapor interfacial tensions. Later, for practical surfaces, Wenzel [75] gave an expression for the contact angle $\cos\theta_w = r\left(\frac{\gamma_{sv}-\gamma_{sl}}{\gamma_{lv}}\right)$, taking surface roughness into account where θ_w is the Wenzel contact angle and r is roughness ratio given as the ratio of actual surface area with roughness to the projected/planar surface area assuming a perfectly flat surface. Again, for surfaces with chemical heterogeneity, Cassie-Baxter contact angle relation was given as $\cos\theta_{CB} = f_1 \cos\theta_1 + f_2 \cos\theta_2$, where f_1 and f_2 are the fractions of the total surface area of the respective surfaces and θ_1 and θ_2 are the respective Young's contact angles on the surfaces. Simple and efficient fabrication techniques including robust and established processes like photolithography [76], plasma etching [77], and particle deposition [78] are used to create micro/nano roughness on a range of substrates including but not limited to PDMS (polydimethylsiloxane), glass, steel, etc. Further, for hydrophobic modification, fluoroalkyl or silyl groups are deposited as they have low surface energy. Specific patterning is obtained using methods like oxygen plasma exposure and ultraviolet (UV) irradiation [79].

A range of simple techniques like surface enhanced Raman spectroscopy (SERS), colorimetry, and blood plasma separation have been carried out using open surfaces. SERS enhances the signals for detection of molecules using Raman spectroscopy by coupling of plasmon surfaces [80]. The nanodendritic structures enhance the Raman signal. Given the superior condensation properties of superhydrophobic surfaces, analytes with concentrations as low as 10^{-18} M have been concentrated and detected on them. SERS is also used for the detection of various miRNAs [81]. Similarly, colorimetry is the simple process of looking at color differences to measure a property. The same has been used for biosensing applications when used with a superhydrophilic spot [82]. Surface acoustic waves (SAWs) have been used for

decades in the electronics industry but recently have also found applications in droplet microfluidics with their unique ability to produce very high fluid accelerations. SAWs have been used in applications like nebulization, microcentrifugation, jetting of droplets, and other applications [58, 83–85]. In essence, surface acoustic waves are simply leakage of surface wave energy generated by a piezo-electric substrate like lithium niobate when actuated into liquid drops at an angle. Similarly, there are other active open-surface techniques, one of them being digital microfluidics, which is being discussed here.

Digital microfluidics (DMF) is one of the most common technology for lab-on-chip systems based upon micromanipulation of discrete droplets [73]. Open-surface digital microfluidics refers to droplet manipulations in an open surface. Different chemical/biological reaction protocols can easily be demonstrated in the same device by reprogramming the order of droplet manipulations. While continuous-flow and liquid-plug microfluidics within microchannels have inherently limited programmability, there is no limit of programmability in open-surface digital microfluidics [86]. Among the different DMF techniques, such as electrowetting-on-dielectric (EWOD) [87, 88], liquid dielectrophoresis (L-DEP) [89, 90], acoustofluidics based on surface acoustic waves [58, 91], and magnetophoresis [92, 93], EWOD and L-DEP use electric fields for droplet manipulation. While EWOD force dominates at DC (Direct Current)/low-frequency regime and effectively actuates conductive liquids [94], L-DEP occurs at high frequency and can actuate dielectric liquids also [95]. The main advantages of such systems using electric fields include: (1) capability to handle extremely low sample volumes, and (2) absence of complicated microstructures for fluid control.

2.3.1 Electrowetting-on-Dielectric

EWOD has emerged as one of the most promising techniques for droplet manipulation in microfluidic devices because it is easily programmable, cost-effective, reconfigurable, and reversible technique [88]. In EWOD, droplets move on a chip surface through electrical modification of the surface hydrophobicity. Patterned electrodes are fabricated onto silicon/glass substrates, and two dielectric layers (polymer dielectrics such as SU8 and Teflon) are added to insulate electricity thus preventing the electrolysis of water [94]. Applying an electrical voltage to one of the electrodes causes the contact line of the drop to spread thereby reducing the contact angle. This change in contact angle is related to the Young-Lippmann equation and depends on the actuation voltage, liquid's surface tension, contact angle, and the device capacitance. Even though this phenomenon is reversible, upon voltage removal, the contact line retracts to a higher contact angle owing to contact angle hysteresis [88]. Electrowetting has been used as an indispensable tool for various lab-on-chip functionalities [96]. Droplet creation, actuation, merging, mixing, and splitting are the fundamental operations [97] that enable biochemical assays on EWOD-based microfluidic platforms. Recently, EWOD has been investigated as a

microfluidic platform for cell manipulation [98], detection of various infectious diseases [99, 100], and for tuning acoustic metamaterials [101].

2.3.2 Liquid Dielectrophoresis

When a dielectric particle is placed in an electric field, electrical charges are induced on the particle/medium interface [102]. The induced charges create dipoles and produce a field that opposes the externally applied electric field. Unlike in a conductor, the induced electric field does not completely cancel the external field. It only reduces the field producing a net electric field inside the dielectric particle. When the dielectric particle is placed in a non-uniform electric field, each half of the dipole experiences unequal Columbic forces, and a net force is imposed on the particle. Depending upon the polarizability of the particle and the suspending medium, it gets attracted/repelled toward/away from the regions of a higher electric field. This electrokinetic phenomenon is termed dielectrophoresis (DEP) or particle dielectrophoresis (p-DEP) and offers controllable, selective, and accurate manipulation of particles depending on their dielectric properties [103]. Fluidic manipulation using DEP is termed liquid dielectrophoresis (L-DEP) [89]. Along with the bulk liquid movement toward the regions of strong electric fields, L-DEP also influences the deformable liquids to have a shape parallel to the electric field lines. In recent years, researchers are also able to manipulate droplets away from strong electric fields by tweaking the medium and the liquid properties [104]. Liquid dielectrophoresis (L-DEP) is an electrokinetic phenomenon in which a dielectric liquid, placed in a non-uniform electric field, moves toward the regions of higher electric field intensity [105]. L-DEP is considered a promising digital microfluidic technique as it offers a rapid and parallel droplet dispensing methodology. Different droplet functionalities such as droplet generation [106], droplet transfer [107], and mixing [90] are achieved when high-voltage (~100–700 V) kilohertz frequency AC signals are applied to the drop, which is placed on dielectric-coated coplanar electrodes. Researchers have demonstrated L-DEP as a potential technique for imparting controlled temperature for biochemical reactions [108] and deicing applications [109]. To minimize the droplet actuation voltage, Froozanpoor et al. [110] introduced a lubricant layer over the electrodes. The surface treatment reduces the contact angle hysteresis associated with the pinning forces at the droplet contact line thus requiring a smaller L-DEP bulk force for droplet manipulation. Through this low-voltage L-DEP platform, one could envisage this technique for energy-efficient self-cleaning applications.

2.4 Acoustofluidics and Applications

With the pioneering works of foremost scientists including Chladni, Kundt, Rayleigh, King, Nyborg, and Gorkov, acoustic manipulation of particles and fluids dating back to the mid-nineteenth century is presently a well-known and established

phenomenon. Be it the acoustic radiation forces in a standing or a traveling wave, or the streaming of the solvent, the underlying physical principles behind a variety of acoustic effects have been thoroughly explained in the literature [37, 111]. Submillimetric wavelengths render acoustic waves tailor-made for microfluidic applications giving birth to *acoustofluidics* [112]—a fusion of acoustics and microfluidics. With the ever-rising research interests in bioanalysis and clinical biology, acoustofluidics is the point of attraction to a wide variety of scientists as it embodies a biocompatible platform offering manipulation of bioparticles purely based on mechanical properties such as size, density, and compressibility. In microfluidic chip, the ultrasonic waves [74] are typically generated in either the bulk (BAW—bulk acoustic wave) of the fluid or on the surface (SAW—surface acoustic wave) of a piezo-electric substrate, actuated by an alternating current supply through the attached piezo-ceramic transducer (PZT) or deposited interdigitated transducer (IDT) electrodes, respectively. Ultrasonic wave technology, which is gentle, non-invasive, simplistic, robust, and label-free, has gathered huge research and clinical interest in the bioanalytical and biomedical community. Several applications [34] ranging from cell/particle sorting, cell/particle separation, cell/particle concentration, bioassays, cell lysis, and PCR (Polymerase Chain Reaction) to mixing, pumping, enrichment, trapping, and nebulization involving microfluidic handling of a cell, particle, drop, bubble, spheroids, and fluids have been extensively reported in the literature. In the present part of the chapter, we briefly discuss the principles behind acoustofluidics and its various applications in detail, particularly emphasizing the manipulation of particles and fluids—classified under the categories of BAW [1], SAW [113], and oscillating bubbles/sharp edges [114].

2.4.1 Bulk Acoustic Waves

BAWs form resonance modes inside microchannels/resonators enabling the generation of standing waves due to reflection occurring between the hard walls of the microchannel. For a fixed channel dimension, the number of pressure nodes and antinodes in the matching layer between the hard walls depends on the frequency of ultrasonic actuation. Higher frequency enables a higher number of nodes, thus subjecting the particle/cell to stronger acoustic radiation force and thus relishing a better control in separation and focusing applications in a continuous manner. Depending on the sign of the acoustic contrast factor of the interfering object, it tends to get attracted either to the pressure node (+) or the antinode (−). As the particles are exposed to acoustic radiation forces, they also get affected by the streaming drag forces induced inside microchannels. The well-established and high-throughput platform of bulk acoustic wave-mediated acoustophoresis has a wide variety of applications, especially in continuous-flow microfluidic systems. Some of the major applications include separation of nanoparticles [115–117], separation of microparticles [118–126], cell focusing [127–129], cell sorting [130], microparticle concentration [131], and ultrasonic enhancement of immunoassays [132]. Separating cells and secreted vesicles under the same conditions is desired but

challenging, and was recently attempted by Dolatmoradi et al. [115] utilizing the difference in acoustic contrast based on varying on-chip thermal environments and cholesterol content of the vesicles. They showed thermally assisted high-efficiency acoustophoretic separation for three vesicle types, different in the cholesterol content. The presence of red blood cells (RBCs) along with the bacteria in a bacteriophage-based luminescent assay greatly reduces the limit of detection and is a bottleneck in the development of detection and diagnostic techniques. Dow et al. [116] proposed a simple, cost-effective acoustic separation-based bacteria detection technique that separated RBCs from the target bacteria cells before the detection step, and hence achieved a 33-fold improved limit of detection. Petersson et al. [124] demonstrated free-flow acoustophoresis, enabling separation of differently sized microparticle suspensions in a continuous manner. Mixed-sized particles under the action of radiation force traversed laterally to different regions of the cross section, and were suitably collected from the corresponding outlets at different channel lengths. By manipulating the density of the suspending medium with the density gradient medium, it was also shown to separate otherwise acoustically inseparable (red cells, platelets, and leukocytes) particles. Multinodal acoustic focusing of cells in a high-throughput parallel flow cytometer was reported by Piyasena et al. [127], which proved to be an improved version of the traditional cytometer. Unlike the conventional flow cytometers, the adopted multinodal approach was shown to exhibit particle focusing in multiple streams with a size range from 7 to 107 μm, and very high throughputs of up to 1 mL/min. A fluorescence-actuated acoustic particle sorter was demonstrated by Jakobsson et al. [130], wherein prefocused target particles based on fluorescence detection were given pulsed actuations, enabling sorting of target particles. The concentration and enrichment of cells from dilute samples is a critical step in many biochemical assays. To overcome the shortcomings of the conventional centrifugation technique, Jakobsson et al. [131] came up with a novel acoustofluidic recirculating system applicable to a wide variety of cells (without affecting viability), enabling a 1000-fold concentration increase with very high throughput and recovery rate.

2.4.2 Surface Acoustic Waves

IDTs enable the generation of SAWs with MHz to GHz frequency range. Traveling SAWs (tSAWs) are typically generated by IDTs deposited on a piezo-electric substrate. The difference in the speed of sound in the elastic substrate and the fluid medium makes the waves leak in the fluid medium at the Rayleigh angle and create pressure perturbations in the medium. Suitable interference of traveling waves can generate standing waves in a SAW setup. As explained in the previous section, the acoustic contrast factor decides the final position of the interfering object in the case of a standing SAW (SSAW) setup also. With further advancements in the field and to overcome certain challenges, tilted angle SSAW (taSSAW) has also proven its importance in recent times. With no strict requirement of hard wall material (glass, silicon, etc.), a SAW setup gets smoothly integrated with the low-cost, much-desired

polydimethylsiloxane-driven microfluidic fabrication. Also, since the acoustic power is directly supplied to the surface of the substrate, here a large amount of energy is directly utilized at the desired location leading to faster fluidic actuation compared to the BAW setup where a portion of the energy is spent only to actuate the entire bulk of the medium. Owing to the above-mentioned advantages, SAW [133–141], SSAW [142–151], tSSAW [152–155], and tSAW [156–159] setups have been extensively utilized among various biomedical and engineering sectors including separation of nanoparticles [142, 151, 152], separation of microparticles [145, 148, 153–155, 157, 159], cell focusing [146, 150], cell sorting [143, 147, 149, 156, 158], nanoparticle concentration [139–141], microparticle concentration [144], fluid mixing [134, 135], pumping [136–138], cell lysis, and PCR [133]. Nucleic acid-based tests (NAT) in diagnostics generally require sophisticated equipment and laboratories to perform thermal cycling and centrifugation for PCR. Reboud et al. [133] demonstrated an apparatus that could potentially enable lab-on-chip PCR by replacing bulky setups with microfluidic chips. They reported a SAW setup with a phononic lattice to carry out lysis of a blood drop; with further heating by switching frequencies and incorporating passive cooling they were able to carry out NAT-on-chip with very high detection capabilities. Using SAW-enabled enhanced mixing, Gracioso Martins et al. [134] reported a 100-fold improved version of a flow injection analysis system with an in-house miniaturized chemiluminescent species photodetection scheme. With contactless liquid-handling technology on a rise, digital acoustofluidics has gathered interest recently. Zhang et al. [137] present a contactless SAW-based contaminant-free liquid-handling technique that allows for digital transport, merging, mixing, and splitting reagents within aqueous droplets, potentially paving way for fluidic processors. High-throughput sorting of rare cells with very high sorting efficiency on a low-cost integrated platform is challenging. Ren et al. [147] recently demonstrated a fluorescence-activated SSAW-based cell sorter that had integrated focusing, fluorescent detection, deflection, and collection unit of target HeLa cells on a single chip by using a combination of straight focusing and focused deflecting IDTs. From the same group, an SSAW setup [144] was utilized to enrich rare cells from larger sample volumes in a continuous manner. Trapping of cells against the flow and furthering clustering at the trapped sites helped concentrate the low-abundance samples. Separating nanoparticles of significance using a single microfluidic chip is the need of the hour in the therapeutic and health diagnostic technologies. Wang et al. [152] developed a two-step separation of exosomes from a whole blood sample on a single microfluidic chip. In the first step, microscale entities like RBCs, white blood cells (WBCs) were separated from nanoscale entities. Further, in the second step, exosomes were separated from other nanometric scaled objects like apoptotic bodies and microvesicles based on size using taSSAW. Nodal planes at a tilted angle remarkably enable highly efficient separation down to the nanometric scale (110 nm).

2.4.3 Oscillating Bubbles/Sharp Edges

Acoustically oscillating microbubbles in a liquid medium can generate acoustic radiation forces and microstreaming vortices giving rise to enhanced fluidic motions enabling rotational manipulation [160], trapping [114] of target entities, transporting particles [161], and mixing applications [162]. Similar to the microbubbles, by controlling the actuation frequency and the geometrical features, the oscillating sharp structures [163] can perform a variety of operations such as pumping [164], mixing [162, 165, 166], rotational manipulation, nanoparticle synthesis [162], spheroid formation [114], cell lysis [167], and microparticle trapping [168]. Trapped microbubbles inside predefined cavities along the channel have shown promise in several applications. In a study by Ahmed et al. [160], the authors showed that acoustically oscillating trapped microbubbles generate microvortices that can rotate single cells and organisms. Oscillating microbubbles combined with sharp-edged structures cause strongly propagating microvortices that enable rapid spheroid [114] (compact cell-collagen aggregates) formation. Acoustically oscillating sharp edges standalone have proven their versatility in a variety of applications. One such study [168] explores cell and particle trapping on sharp structures. It was found that depending on the properties of cells/particles, the tip of the sharp edge can attract or repel particles. Besides this, oscillating bubbles [161, 162] and sharp edges [162, 164, 166] have been shown to enhance mixing and enable pumping in microfluidic systems.

2.5 Lab/Organ-on-Chip for Drug Testing Application

2.5.1 Limitations of Traditional Drug Development

More recent data show that the duration time for the drug development is becoming extensively long and costly, which typically requires 12 years. The normal expense for developing each clinically accessible drug is more than $1.7 billion and also under the state of high research expenses, the number of recently approved medications is very low every year [169, 170]. Nowadays, the utilization of physiologically based pharmacokinetic (PBPK) modeling and simulation approaches has made huge advances in foreseeing the key pharmacokinetic (PK) boundaries from human in vitro study. This in turn developed as a significant tool in drug development and regulation [171–173]. However, such methodologies neglect to incorporate organ-specific differentials in drug clearance, distribution, and ingestion, which are caused by the distinctive cell uptake, transport, and metabolism [171]. One mainly depends on in vitro cell culture stages and in vivo creature models, and another alludes to the drug administration on human bodies [169]. In addition, traditional methods gradually cannot satisfy the growing demand for new and effective drugs. What is more, during using traditional disease models, drug development will be time-consuming because of those inaccuracies caused by different species' genes as well as raising ethical concerns. In a word, traditional methods

cannot reproduce the functions of the complicated biological structures, preventing people from getting timely and accurate analyses about diseases. These results have brought a heavy burden on the health care sector in the whole society and subsequently overwhelm the drug development progress [174].

2.5.2 Introduction to Organ-on-Chip (OOC)

The increasing accessibility to point-of-care health devices has opened the doors for the pharmaceutical industries to broader their market potentials. Such niche emerging markets are crucial for pharma companies. One among the several challenges is its increasing costs on the models that are genetically engineered to express supportive growth factors that are more human-like in terms of genetic and immunological background and the microbiome. This made the drug discovery researchers take imperative steps for new testing approaches that can make dependable predictions of drug efficacy and safety in humans. Further, for drug development, animal models are acquiring more human-like genetic backgrounds, immune systems, and microbiome for better results [175]. Recently, researchers have found that by micro-engineering cell culture models, an effective solution to the above-mentioned problem can be reached. These models recapitulate physiologically important functions of one or more human tissues or organs and their interactions. It involves microfabrication and microfluidic technologies to precisely control the cellular microenvironment for a better representation of various human physiological situations. The advanced progress has emanated to the development of promising microdevices, known as organs-on-chips (OOCs), which can replicate both the biology and physiology of human organs in vitro [176–179].

2.5.3 Organ-on-Chip Technology for Drug Development

Organs-on-chips are micro-engineered biomimetic systems that reconstruct the functional miniaturized units of living human organs with three-dimensional (3D) microfluidic cell culture chip [180, 181]. They are usually composed of transparent 3D polymeric microchannels arranged by live human cells and duplicate three vital aspects of a complete organ: (1) the 3D microarchitecture defined by the spatial distribution of multiple tissue types; (2) functional tissue–tissue interactions and interfaces; and (3) dynamic mechanical and biochemical stimuli found in particular organs. These dedicated chips could be used as specialized in vitro models for simulations, mechanistic studies, and pharmacological adjustments of complicated biological processes, which will be powerful tools for drug delivery, analyte-specific monitoring, and medical diagnostics via more precise investigations and therapies [182, 183]. The goal of organ-on-chip is not about building an intact living organ but it is about synthesizing the most basic functional units of tissues and organs. The simplest chip is a single, perfused microfluidic chamber containing a cultured cell, such as endothelial cells, that functions as a certain tissue type. As for

other more complex designs, there are more than one microchannels connected by porous membranes, with different cell types arranged on opposite sides, to reconstruct the interfaces between different tissues. These systems involve physical forces, including physiologically related fluid shear stress and mechanical compression, and they also allow the analysis of responses of a specific organ, like recruitment of circulating immune cells, in reaction to drugs, toxins, or other environmental disturbances [184]. In this section, we have summarized the recent progress in organ-on-chip. Firstly, we described the characteristics of organ-on-chip, the drawbacks of existing traditional dug screening methods, and discussions regarding the existing organ-on-chip studied with human tissues and organs in vitro. Secondly, we compared traditional models of drug discovery and novel organ-on-chip technology that shows advantages in mimicking complex in vivo conditions and the analysis of complicated physiology issues, and organs-on-chips' focus on using human-induced pluripotent stem cells (hiPSCs) to predict human response to any treatments, which contributes to the development of personalized drugs. And we introduce studies performed on human-on-chip and conclude the section by discussing the challenges for human-on-chip to meet the needs of the patient for drug development and clinicians.

With the advancement in modern techniques, various companies in the pharmaceutical industry have shown exponential achievements in developing more effective and low-cost drug discovery models in recent years. And relying on this organ-on-chip technology, the integrated organ systems, such as the heart, lungs, liver, and so on, are studied to provide an optimal in vitro model for drug discovery [185–189]. For instance, in the lung organ-on-chip, alveolar tissue and blood arteries are essential for the investigation of pulmonary drug discovery [190–193]. In this way, the current human models are being studied for infection pathogens and drug screening. The alveoli, the basic functional unit of the lung, is mimicked by a cyclic actuation of gas permeation surface [189]. Scientists are utilizing these chip models to resolve past issues, such as lack of association between the immune system and lung tissue, which have added convincing leap forward and could change the possibilities for discovering promising future medicines for lung infections [20, 24]. For example in the case of lung-on-chip, in 2017, Benam and other researchers developed a microfluidic device in which interfaces are separated by mucociliary bronchiolar epithelium, through designing a tiny airway, an air-blood separation barrier, to investigate the physiological and pathophysiological systems. This model eludes the new fundamental constriction of the airway mucosa and mimics the roles of the real interactive immune system and endothelial tissues. Also, those tissues experience shear stresses from the blood stream, while actuating the actual airway. This refined lung-on-chip model makes it practical to study critical lung diseases identified with immune systems, similar to the comorbidities of chronic obstructive pulmonary disease (COPD) and asthma [194]. Especially, several lung-on-chip models add to the development of the biologically inspired OOCs; few other organ-on-chip models are shown in Fig. 2.2 [181, 189, 194].

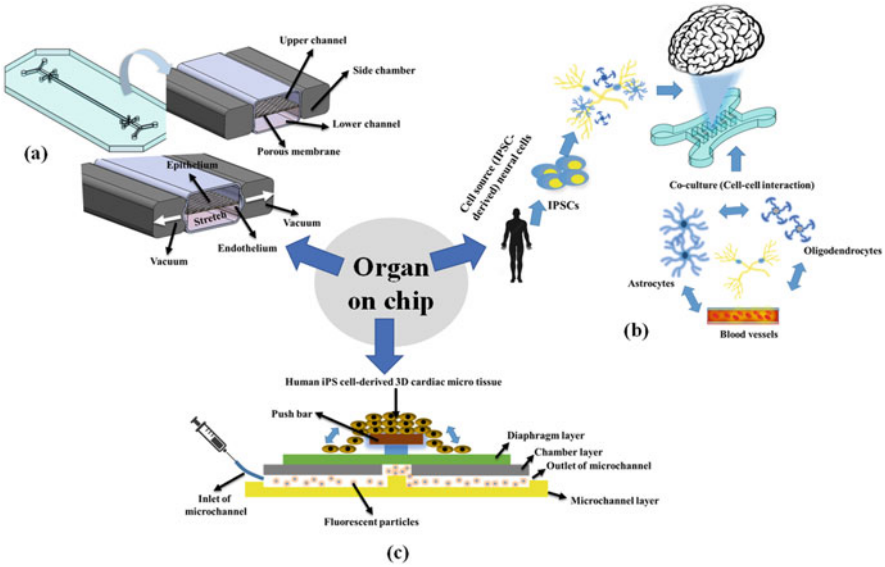

Fig. 2.2 The organ-on-chip models: (**a**) lung on chip [181], (**b**) brain on chip [195], and (**c**) eye-on-chip [196]

2.5.4 Future Scope: Human-on-Chip

Despite significant progress in developing organ-on-chip and micro-engineered tissues, effective drug toxicity testing actually should involve the implementation of each organ and its interactions. Thus, the complete organs must be functionally integrated into the human body in the future, establishing a fully functionalized microfluidic circulatory system. Still, there is a lot of work to be done in the development of complex and complete models that recapitulate the metabolism and physiology of the entire organs. Thus, researchers have further put forward the concept of "human-on-chip" models by interconnecting individual chambers composed of the whole organ model. Each compartment contains different cell types representing different human organs and is connected by the microfluidic system in a microfabrication device. It can be approximated to the actual physiological fluid-flow conditions, realistic size ratios, and multi-tissue interactions that may help in developing physiological pharmacokinetic models. Biomimetic microsystems representing different organs are integrated into a microfluidic device and connected in a physiologically applicable way to simulate complicated interactions and dynamic drug processes of absorption, distribution, metabolism, and excretion for evaluation of drug efficacy and toxicity [197]. The main requirement for organ-on-chip systems in the future is to develop major new drug screening and discovery methods to accurately characterize the response of these microchips to pharmacological modulation and to verify their ability to foresee the response of drugs with well-defined characteristics of human nature. A key concern will be to determine the

best source of human cells for accurate response in the body. The second important issue lies in combining the scale-up of complex techniques with the integrated engineering systems, such as micro-engineered organ simulators with sensors to detect and measure various light, chemical, electrical, and mechanical signals from cells to analyze their structures and functions [198].

2.5.5 Challenges and Perspective

Even though researchers have achieved great progress in the organ-on-chip technology, which has become a significant tool for developing in vitro human disease models for drug screening and development, several challenges are yet to be addressed with extensive research to get more reliable outcomes. One of the major problems is from a material perspective; PDMS is used in all the devices due to its high optical clarity, gas permeation, and superior biocompatibility. Despite all these advantages, it has very poor chemical resistance toward the organic solvents, which has a major disadvantage while functionalizing the surface. It is also capable of absorbing hydrophobic molecules, chemical reagents, and fluorescent dyes. Therefore, it will influence the drug testing that involves very precise reagents of potential drug screening. Another problem involves ECM (Extra cellular matrix)-coated PDMS membrane for the tissue–tissue interface that can develop rather different transport than the natural membrane. Another challenge is with respect to the multisensory system. Although few research groups have achieved the microfluidic device that mimics organ-specific functions, several integrated aspects of organs in the case of the multisensory platform are yet to be investigated. However, most of the studies highlight the effects of a particular organ function, but very few researchers have addressed the integrated effects of combining multiple working organ-on-chip devices. In the future to attain this, firstly it requires precise devices that are well developed with active interfaces to link multiple organs-on-chips to develop a human-on-chip. Secondly, more suitable device materials and fabrication methods must be identified for disposable mass production chips. Finally, we predict that our future organ-on-chip devices must deliver high-throughput analysis for commercial applications. To reach the level of a live human-on-chip, the media and physiological conditions must be optimized for better organ–organ interaction [199].

2.6 Biosensing Applications

Biosensors are nowadays an essential part of advanced health care technology. Biosensors are being used in disease detection, treatment, disease prognosis monitoring, treatment monitoring, etc. The advantages of miniaturized biosensors have driven the research community toward the evolution of new state-of-the-art technologies. The simplicity in operation, sensitivity, accuracy, portability, ability to perform multiple analyses simultaneously, ability to interface with different

systems, etc. have given rise to point-of-care diagnosis platforms. Most disease diagnosis technologies are based on lab tests that require multiple laborious procedures, skillful operations, sufficiently high sample volume, and reagents and consume a lot of time. Lab-on-chip is a revolutionary technology that integrates all such laboratory functions in one single compact chip and utilizes a very less amount of samples/reagents (in range of picoliters to milliliters). Also, it allows us a massive amount of parallelization for high-throughput screening. The invention of micro-technology allows us to develop extremely small-sized sensors, actuators, and sample processing platforms. Microfluidics is a technology providing platform for the precise handling of extremely small amounts of fluids, which can be chemicals, reagents, biofluids, etc. Microfabrication technology enables the production of small microchannels, incubation regions, reaction chambers, etc. We can further integrate biosensors and microactuators. Due to excellent control over samples, we can perform very efficient screening and detection. Response time is faster as parameters like sample volume to surface area ratio, reaction time, diffusion time, etc. are significantly reduced. Various microfluidic platforms have been developed for chemical and biological applications. Biological applications mainly involve the detection of biomolecules, drug testing, drug delivery, etc. Microfluidics-based biosensors can be classified in various ways based on:

- Technology domain (continuous-flow microfluidics, droplet microfluidics, digital microfluidics, etc.)
- Detection/processing techniques (optical, acoustic, electrical, etc.)
- Types of biomolecule (enzymes, aptamers, antibodies, etc.)

Here, we will see different biosensing applications based on types of biomolecules.

2.6.1 Antibody-Based Detection

In this method, the detection target is a specific antigen/surface protein present on the surface of the biocomponent. Every antigen has its unique specific antibody, which can be used for detection. This antibody is conjugated with a signaling compound that can help us in detection. Conjugating antibody with fluorescent molecule [69] is a very popular technique for detection. The fluorescent molecule can be either dyes or nanoparticles like quantum dots (QDs), polymer dots (PDs), upconversion nanoparticles (UCNPs), gold nanoparticles (AuNPs), etc. [200]. In some works, antibodies were conjugated with magnetic beads [201, 202], which uses a magnetic field integrated with an appropriate microchannel design for detection and isolation. Some works have used acoustic forces to immobilize the antibody conjugated biomolecules [203]. In one of the works, antibodies were immobilized inside the channel via antibody-coated micro-posts, which helped to immobilize target biomolecules in controlled laminar flow [204]. Antigen-antibody detection via localized surface plasmon resonance (LSPR) has shown a promising future as it

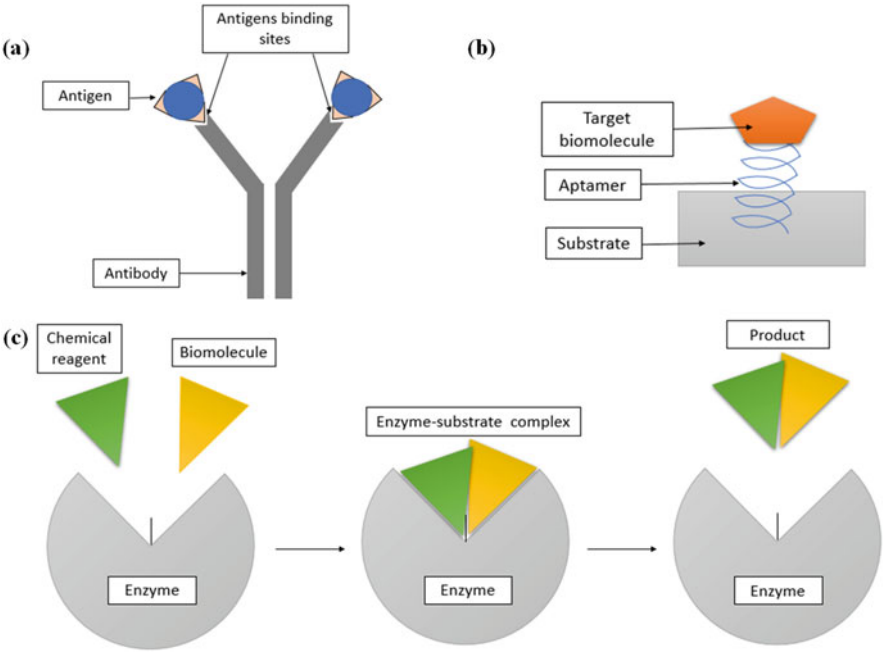

Fig. 2.3 (**a**) Antigen–Antibody interaction; (**b**) Aptamer immobilization to capture and detect biomolecule; and (**c**) Enzyme action

can detect a nanomolar concentration of antigens within a few minutes from sample volume as small as 10 μL [205] (Fig. 2.3).

2.6.2 Aptamer-Based Detection

Aptamers are RNA or DNA oligonucleotide or peptide molecules that can bind to very specific targets. Aptamers can be artificial, chemically synthesized, or they can be naturally occurring. Artificial aptamers are also termed "chemical antibodies." Production of aptamers is in vitro synthesis based (by Systematic Evolution of Ligands by Exponential enrichment [SELEX]) and not dependent on living being's immune system response like antibodies. Aptamer-based detection is gaining popularity because of its relative chemical and synthetic stability, short length, enhanced functionality, specificity, etc. Due to aptamers, it has been possible to detect toxic biomolecules/compounds, which are impossible to detect with antibody technology as the immune system fails to produce antibodies against them. Similar to antibody-based detection, it uses optical- [206], electrochemical- [206], and FET (Field effect transistor)-based [206, 207] detection methods. The optical-based detection method mostly involves the detection of fluorescence molecules conjugated with aptamers. The electrochemical-based detection method works on the principle of change in the

electrical property of the sensor such as electrical current, electrical impedance, charge accumulation, etc. due to sample. Several works reported immobilization of aptamers [208] on electrodes, carbon nanotubes, and FETs, which can detect biomolecule in microchannel.

2.6.3 Enzyme-Based Detection

Enzymes are proteins that catalyze biochemical reactions. The enzyme helps in the reaction of target analytes to produce chemical signals that can be converted into other forms using transducers. The advantage of enzyme-based biosensors is high selectivity/specificity and sensitivity. The detection method mostly involves electrochemical detection [209]. The chemical reaction in the sample leads to change in its properties like the number of free charges/ions, the conductivity of the solution, etc., which can further be detected using detection techniques like potentiometric, impedimetric, amperometric, etc. The most common electrochemical enzyme biosensors involve redox reactions, for example, lactate biosensors [210], glucose biosensors [211], etc., which are widely used biosensors. In these methods, oxidase compound (lactate oxidase [LOx], glucose oxidase) reacts with target analyte in the presence of oxygen, causing oxidation of other compounds, which leads to reduction of another compound in assay that can further be detected. Some work demonstrated the detection of blood gasotransmitters like H_2O_2 [212], NO [213], etc. Detection techniques can also involve optical detection methods. Some of the works have demonstrated the detection of blood constituents like gasotransmitters as H_2S [214], H_2O_2 [215], and circulating tumor cells (CTCs) [216]. Detection of CTCs is based on their lactic acid metabolism. CTCs are observed to secrete lactic acid by the phenomenon known as the Warburg effect [217] during metabolism. The cell is first encapsulated in a droplet. Cell secretes lactic acid inside the droplet, which is further detected using lactic acid detection assays or pH-sensitive dyes.

2.6.4 Challenges

As microfluidics technology is under developing stage, it has some limitations in biosensing applications like:

- Needs very high-cost sophisticated device fabrication technology.
- Lack of standardization in detection techniques.

2.7 Cell Manipulation

In the field of cellular biology, the analysis of cells and intracellular components is of utmost priority. In the 1950s, due to the development of microscopy techniques, it became possible to analyze and study the behavior of intracellular components.

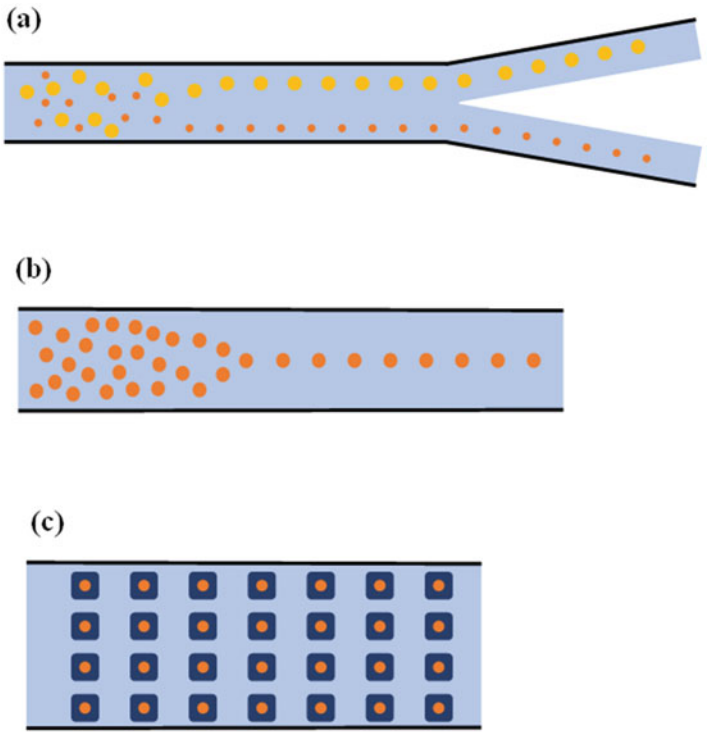

Fig. 2.4 (**a**) Cell sorting into two channels; (**b**) Cell focusing into a single central stream; and (**c**) Cell trapping in the specified region

However, understanding the heterogeneity of each cell is crucial. Various conventional techniques performed in microwells, glass slides, and Petri dishes do not help in single-cell analysis. With the advancement of microfluidic technologies in the past 30 years, it is possible to manipulate and analyze biological molecules to a single-cell level. Since microfluidic techniques are performed in tiny closed microchannels (on-chip), which range in the order of tens to hundreds of microns, it is very easy to avoid contamination and a very low sample volume is required. Analysis of cells to single-cell level showed promising results in diagnostic applications. The use of microfluidic cell manipulation techniques in biomedical research along with the current micro-electromechanical systems (MEMS) technologies helps in miniaturized diagnostic devices. Cell manipulation mainly involves focusing, sorting, transportation, encapsulation, trapping, isolation, and rotation. Cell lysis can also be done on chip to rupture the cell walls and extract the intracellular components for further analysis or DNA amplification. Numerous manipulation techniques are implemented in microfluidic techniques. Depending on the types of forces applied, these techniques are classified into mechanical, optical, electrical, magnetic, and others. In this section, we will discuss how these methods are used in cell manipulation in detail (Fig. 2.4).

2.7.1 Mechanical Manipulation

Most of the mechanical manipulation techniques are passive. This provides a major advantage when compared to other techniques. Sorting of cells for analysis, counting, or culture is the main application of on-chip mechanical manipulation. These can be achieved by narrowing down the channels, modifying the surface of the channels by reactive coatings [218], or by creating constrictions in the channel's path such as microwells [219], microgrippers [220], etc. Y. Tang et al. [221] used microfilter-based devices having conical-shaped microhole arrays to capture circulating tumor cells (CTCs) from the blood. They validated this by filtering CTCs spiked in blood and also detecting CTCs in cancer patients' blood samples. They cultured the cells on the same filter and achieved cell viability of nearly 95%. Microtraps can be created to parallel the immobilization of cells with high occupancy inside microchannels. Di Carlo et al. [222] demonstrated the trapping and culturing of HeLa cells in U-shaped microtraps. Kim et al. [223] demonstrated the isolation of cells of different sizes using an S-shaped microtrap array chip based on a size-based cell bandpass filter. Microwells in the order of size of the cell can trap or immobilize the cells. When the cell sample is flown into microchannels, the cells sediment and settle down in the microwells. The redundant cells are washed away. The efficiency of these passive techniques is low, and it also depends on the cell concentration, active surface area, and flow rates. Micropatterning is another passive technique used to trap cells in arrays using chemical surface patterns with cell adhesive spots surrounded by cell repellant spots. Patterns in different shapes can be used to understand cell behavior. Yang et al. [224] captured CTCs from blood sample by coating anti-EpCAM (Epithelial cell adhesion molecule) to hydrodynamically optimized triangular micropillars. Similar to trapping of cells in microwells, the cells can be encapsulated into femtoliter- to nanoliter-sized droplets. Droplet microfluidics is of primary interest in cell biology due to very low chances of contamination and faster mixing inside droplets. The droplet size and frequency can be controlled using interfacial tension, flow rates, and viscosities [225]. R. Gaikwad and Sen [69] encapsulated cancer cells (HeLa and DU145) inside droplets, and the fluorescence signals from the target cells were measured and sorted using suitable electronics. Since the frequency of the droplets ranges from a few droplets per second to thousands of droplets per second, high-throughput detection is an additional advantage of this technique. The concept of flow cytometry can be performed using microfluidics based on the hydrodynamic manipulation of cells. Hydrodynamic focusing of cells and the use of sheath fluid help in aligning cells for a single line of detection. It is advisable to eliminate sheath fluid and hydrodynamic forces in portable analysis and diagnostics. The simplest way to avoid this is to use microchannels having a size comparable to the size of cells. Anne Y. Fu et al. [226] demonstrated a disposable microfabricated fluorescence-activated cell sorter (μFACS) for sorting *Escherichia coli* cells using a 4 μm × 3 μm microchannel. Since a high concentration of cells leads to clogging in narrow microchannels, only diluted cell samples can be used, which in turn decreases the throughput. Clogging usually happens if the cell to channel dimension ratio is more than 0.33 [227]. Instead of

only using narrow channels, the deformability of the cell or modifying the geometry of the microchannel can reduce the probability of cells getting clogged [228, 229]. Many attempts have been made to eliminate the sheath flow by using aerodynamic cell manipulation, but the throughput using these techniques is very low [230]. Modifying the geometry of the microchannels can induce internal forces such as hydrophoretic or inertial forces, which can also be used to avoid the sheath flow [64, 231]. These forces depend on the size difference between the cells and the microchannels. S. Hazra et al. [5] demonstrated a passive non-inertial lift-induced migration and sorting of cancer cells (EpCAM+ and CD45−) from PBMCs in a co-flowing aqueous two-phase system. P. Sajeesh et al. [232] demonstrated sorting based on the size and deformability of the cells.

2.7.2 Electrical Manipulation

Dielectrophoresis is the most used technique for sorting cells using electrical manipulation. It is a phenomenon where the force is exerted on a dielectric particle when subjected to a non-uniform electric field. Dielectrophoretic manipulation of cells depends on the size of the cells, dielectric properties of the cells, surrounding medium, and gradient in the electric field. It is categorized into positive DEP and negative DEP. In positive DEP, the cells tend to move toward the region of a strong electric field; in negative DEP, cells tend to move toward the region of a weak electric field. Anas Alazzam et al. [233] demonstrated the separation of cancer cells, specifically green fluorescent protein labeled MDA-MB-231, from normal blood samples. They used a set of planar interdigitated transducer electrodes deposited on the surface of the glass wafer that is slightly protruded into the microchannel at one side. Unlike planar electrodes on the bottom surface of the microchannel, Lisen Wang et al. [234] used interdigitated electrodes on the side walls to manipulate the cells in the lateral direction as well.

2.7.3 Optical Manipulation

Optical manipulation uses optical force induced by a highly focused laser beam on particles or cells. To manipulate a target object, this technique uses the difference in gradient optical force that pulls the target particles and scattering force that repels the target particles. Cell sorting is the most widely used application of optical manipulation. When a fluorescent particle is passed through an electrostatic detection system, the fluorescent intensity is measured and manipulated. This technique is known as fluorescence-activated cell sorter (FACS). The pre-determined target cells can be switched or sorted using optical forces based on their size, refractive index, or light absorption. Yeast cells and human embryonic stem cells are sorted using this technique [235]. Optical tweezers are another technique used to manipulate particles based on their refractive index. It was first reported by Palmer et al. [236]. Under a highly focused laser beam, a high refractive index object could be dragged to the

center of the beam more than that of a lower one. An optical tweezer-based laser can manipulate objects in the order of nanometers to tens of microns. Zheng Zhang et al. [237] trapped *E. coli* for at least 30 min without affecting the cell viability. The typical force applied in this technique is in the order of pico-Newtons. So, at very high flow rates, it becomes difficult for the tweezers to deviate the cells from their streamlines. Optical-induced dielectrophoresis (ODEP) is another technique used to manipulate the cells. It is similar to traditional DEP technology. The difference between them is the technique in which a non-uniform electric field is generated. Unlike traditional DEP manipulation, ODEP does not need prefabricated electrodes. The digital micromirror device (DMD) projectors are used to project light patterns on the microfluidic device, which acts as virtual electrodes. Tzu-Keng Chiu et al. demonstrated CTCs' isolation from leukocytes using ODEP [238]. ODEP is simpler than the optical tweezers and opaque objects can be manipulated using this technique.

2.7.4 Magnetic Manipulation

Magnetic manipulation is the technique in which cells are manipulated using external magnetic forces. It is categorized into two, one is positive magnetophoresis and the other is negative magnetophoresis. In positive magnetophoresis, magnetic particles are attached to the cells; when these are subjected to the magnetic field, the particles deviate their path or get trapped at the required location. Most commonly used super-paramagnetic beads are in the range of 10–100 nm in diameter, which does not affect cell viability or cellular functions. Most of the cells are diamagnetic. To avoid the tagging of cells with magnetic particles, negative magnetophoresis can be used. Here, the cells are suspended in a magnetic medium or ferrofluids. In the case of positive magnetophoresis, the cells with intrinsic magnetic properties like red blood cells and magnetotactic bacteria can be easily manipulated [239]. If the cells do not show any intrinsic magnetic properties, they need to be tagged with magnetic particles. Tagging them with magnetic particles will increase the cost and also requires longer incubation times for tagging and several wash steps; whereas negative magnetophoresis is a label-free technique where the cells are suspended in a magnetic medium. When the cells suspended in ferrofluids are subjected to external magnetic force, the cells move toward the region of a minimum field in the non-uniform magnetic field. Diamagnetophoresis is a specific technique used to separate cells of different sizes. Fengshan Shen et al. [240] used this technique to sort U937 cells (human histolytic lymphoma monocytes) from red blood cells based on the difference between the magnetic forces applied on cells adjusted by the magnetic susceptibility of the surrounding medium. Focusing of cells inside a microchannel can be achieved by creating a local magnetic field minimum using a pair of magnets on either side of the channel. Rodríguez-Villarreal et al. [241] demonstrated the focusing of HaCaT cells using the repulsion forces exerted by a pair of magnets. Jian Zeng et al. [242] concentrated polystyrene particles and yeast cells in ferrofluid using negative magnetophoresis.

2.7.5 Other Manipulation

Forces induced by surface acoustic waves and bulk acoustic waves can also be used in manipulating the cells. These techniques are explained in detail in Sect. 2.3 of this chapter. Xiaoyun Ding et al. [143] demonstrated sorting of white blood cells into five different outlets by varying the frequencies of the applied standing surface acoustic waves. Maria Antfolk et al. [119] separated breast cancer cells (MCF 7) from white blood cells using bulk acoustics. Ivo Leibacher et al. [168] demonstrated the trapping of yeast cells at microfluidic sharp corners using acoustophoresis.

2.8 Conclusion

In this chapter, we have discussed the basic processes and techniques used in microfluidics. The techniques are divided based on operating platforms as in-channel and open-surface techniques and further based on the mode of actuation as active and passive techniques. Further, a detailed discussion on some of the key applications in microfluidics has been done. Firstly, acoustofluidic applications using both bulk and surface acoustic waves to manipulate, trap, and sort particles and cells are discussed. Then the biosensing applications including early detection of diseases and prognosis monitoring are discussed along with detection classification based on antibody, aptamer, and enzymes. Further, discussions on lab/organ-on-chip and how they can be used for better drug testing applications have been carried out. We have also focused on the cell manipulation techniques using active and passive actuation to better understand the extent to which microfluidics can be used to manipulate small entities.

References

1. Leibacher I, Reichert P, Dual J (2015) Microfluidic droplet handling by bulk acoustic wave (BAW) acoustophoresis. Lab Chip 15:2896–2905
2. McGrath J, Jimenez M, Bridle H (2014) Deterministic lateral displacement for particle separation: a review. Lab Chip 14:4139–4158
3. Bijarchi MA, Dizani M, Honarmand M, Shafii MB (2021) Splitting dynamics of ferrofluid droplets inside a microfluidic T-junction using a pulse-width modulated magnetic field in micro-magnetofluidics. Soft Matter 17:1317–1329
4. Majhy B, Singh VP, Sen AK (2020) Understanding wetting dynamics and stability of aqueous droplet over superhydrophilic spot surrounded by superhydrophobic surface. J Colloid Interface Sci. https://doi.org/10.1016/j.jcis.2020.01.056
5. Hazra S et al (2019) Non-inertial lift induced migration for label-free sorting of cells in a co-flowing aqueous two-phase system. Analyst 144:2574–2583
6. Sajeesh P, Sen AK (2014) Particle separation and sorting in microfluidic devices: a review. Microfluid Nanofluid 17:1–52
7. Zhou J, Papautsky I (2020) Viscoelastic microfluidics: progress and challenges. Microsyst Nanoeng 6:113
8. Convery N, Gadegaard N (2019) 30 Years of microfluidics. Micro Nano Eng 2:76–91

9. Cui P, Wang S (2019) Application of microfluidic chip technology in pharmaceutical analysis: a review. J Pharm Anal 9:238–247
10. Gabriel EFM, Lucca BG, Duarte GRM, Coltro WKT (2018) Recent advances in toner-based microfluidic devices for bioanalytical applications. Anal Methods 10:2952–2962
11. Nguyen NT, Hejazian M, Ooi CH, Kashaninejad N (2017) Recent advances and future perspectives on microfluidic liquid handling. Micromachines 8:186
12. Bai Y et al (2018) Applications of microfluidics in quantitative biology. Biotechnol J 13: e1700170
13. Oakey J, Allely J, Marr DWM (2002) Laminar-flow-based separations at the microscale. Biotechnol Prog 18:1439–1442
14. Yamada M, Nakashima M, Seki M (2004) Pinched flow fractionation: continuous size separation of particles utilizing a laminar flow profile in a pinched microchannel. Anal Chem 76:5465–5471
15. Berendsen JTW, Eijkel JCT, Wetzels AM, Segerink LI (2019) Separation of spermatozoa from erythrocytes using their tumbling mechanism in a pinch flow fractionation device. Microsyst Nanoeng 5:24
16. Park JS, Jung HI (2009) Multiorifice flow fractionation: continuous size-based separation of microspheres using a series of contraction/expansion microchannels. Anal Chem 81:8280–8288
17. Zeng L, Balachandar S, Fischer P (2005) Wall-induced forces on a rigid sphere at finite Reynolds number. J Fluid Mech 536:1–25
18. Alghalibi D, Rosti ME, Brandt L (2019) Inertial migration of a deformable particle in pipe flow. Phys Rev Fluids 4:104201
19. Yoon DH et al (2009) Size-selective separation of micro beads by utilizing secondary flow in a curved rectangular microchannel. Lab Chip 9:87–90
20. Russom A et al (2009) Differential inertial focusing of particles in curved low-aspect-ratio microchannels. New J Phys 11:75025
21. Duraiswamy S, Yung LYL (2021) Dean migration of unfocused micron sized particles in low aspect ratio spiral microchannels. Biomed Microdevices 23:1–16
22. Huang LR, Cox EC, Austin RH, Sturm JC (2016) Continuous particle separation through deterministic lateral displacement. Science 304:987–990
23. Zhang Z, Henry E, Gompper G, Fedosov DA (2015) Behavior of rigid and deformable particles in deterministic lateral displacement devices with different post shapes. J Chem Phys 143:243145
24. Davis JA et al (2006) Deterministic hydrodynamics: taking blood apart. Proc Natl Acad Sci U S A 103:14779–14784
25. Beech JP, Holm SH, Adolfsson K, Tegenfeldt JO (2012) Sorting cells by size, shape and deformability. Lab Chip 12:1048–1051
26. Quek R, Le DV, Chiam KH (2011) Separation of deformable particles in deterministic lateral displacement devices. Phys Rev E Stat Nonlinear Soft Matter Phys 83:1–7
27. Loutherback K et al (2010) Improved performance of deterministic lateral displacement arrays with triangular posts. Microfluid Nanofluid 9:1143–1149
28. Gascoyne PRC, Vykoukal J (2002) Particle separation by dielectrophoresis. Electrophoresis 23:1973–1983
29. Pesch GR, Du F (2021) A review of dielectrophoretic separation and classification of non-biological particles. Electrophoresis 42:134–152
30. Yaman S, Anil-Inevi M, Ozcivici E, Tekin HC (2018) Magnetic force-based microfluidic techniques for cellular and tissue bioengineering. Front Bioeng Biotechnol 6:192
31. Kersaudy-Kerhoas M, Dhariwal R, Desmulliez MPY, Jouvet L (2010) Hydrodynamic blood plasma separation in microfluidic channels. Microfluid Nanofluid 8:105–114
32. Kim J, Massoudi M, Antaki JF, Gandini A (2012) Removal of malaria-infected red blood cells using magnetic cell separators: a computational study. Appl Math Comput 218:6841–6850

33. Adams JD, Kim U, Soh HT (2008) Multitarget magnetic activated cell sorter. Proc Natl Acad Sci U S A 105:18165–18170
34. Laurell T, Petersson F, Nilsson A (2007) Chip integrated strategies for acoustic separation and manipulation of cells and particles. Chem Soc Rev 36:492–506
35. McGloin D (2006) Optical tweezers: 20 years on. Philos Trans R Soc A Math Phys Eng Sci 364:3521–3537
36. Dao M, Lim CT, Suresh S (2003) Mechanics of the human red blood cell deformed by optical tweezers. J Mech Phys Solids 51:2259–2280
37. Bruus H (2012) Acoustofluidics 7: The acoustic radiation force on small particles. Lab Chip 12:1014–1021
38. Hoque SZ, Sen AK (2020) Interparticle acoustic radiation force between a pair of spherical particles in a liquid exposed to a standing bulk acoustic wave. Phys Fluids 32:072004
39. Hoque SZ, Nath A, Sen AK (2021) Dynamical motion of a pair of microparticles at the acoustic pressure nodal plane under the combined effect of axial primary radiation and interparticle forces. J Acoust Soc Am 150:307–320
40. Sohrabi S, Kassir N, Keshavarz Moraveji M (2020) Droplet microfluidics: fundamentals and its advanced applications. RSC Adv 10:27560–27574
41. Link DR, Anna SL, Weitz DA, Stone HA (2004) Geometrically mediated breakup of drops in microfluidic devices. Phys Rev Lett 92:4
42. Surya HPN, Parayil S, Banerjee U, Chander S, Sen AK (2015) Alternating and merged droplets in a double T-junction microchannel. Biochip J 9:16–26
43. Hatch AC, Patel A, Beer NR, Lee AP (2013) Passive droplet sorting using viscoelastic flow focusing. Lab Chip 13:1308–1315
44. Umbanhowar PB, Prasad V, Weitz DA (2000) Monodisperse emulsion generation via drop break off in a coflowing stream. Langmuir 16:347–351
45. Utada AS et al (2005) Monodisperse double emulsions generated from a microcapillary device. Science 308:537–541
46. Jayaprakash KS, Sen AK (2019) Droplet encapsulation of particles in different regimes and sorting of particle-encapsulating-droplets from empty droplets. Biomicrofluidics 13:034108
47. Zhu P, Wang L (2017) Passive and active droplet generation with microfluidics: a review. Lab Chip 17:34–75
48. Ménétrier-Deremble L, Tabeling P (2006) Droplet breakup in microfluidic junctions of arbitrary angles. Phys Rev E Stat Nonlinear Soft Matter Phys 74:1–4
49. Tan YC, Fisher JS, Lee AI, Cristini V, Lee AP (2004) Design of microfluidic channel geometries for the control of droplet volume, chemical concentration, and sorting. Lab Chip 4:292–298
50. Cho SK, Moon H, Kim CJ (2003) Creating, transporting, cutting, and merging liquid droplets by electrowetting-based actuation for digital microfluidic circuits. J Microelectromech Syst 12: 70–80
51. Jung JH, Destgeer G, Ha B, Park J, Sung HJ (2016) On-demand droplet splitting using surface acoustic waves. Lab Chip 16:3235–3243
52. Christopher GF et al (2009) Coalescence and splitting of confined droplets at microfluidic junctions. Lab Chip 9:1102–1109
53. Xu B, Nguyen N-T, Neng Wong T (2012) Droplet coalescence in microfluidic systems. Micro Nanosyst 3:131–136
54. Niu X, Gulati S, Edel JB, Demello AJ (2008) Pillar-induced droplet merging in microfluidic circuits. Lab Chip 8:1837–1841
55. Bremond N, Thiam AR, Bibette J (2008) Decompressing emulsion droplets favors coalescence. Phys Rev Lett 100:1–4
56. Hemachandran E, Laurell T, Sen AK (2019) Continuous droplet coalescence in a microchannel coflow using bulk acoustic waves. Phys Rev Appl 12:1
57. Sesen M, Fakhfouri A, Neild A (2019) Coalescence of surfactant-stabilized adjacent droplets using surface acoustic waves. Anal Chem 91:7538–7545

58. Sudeepthi A, Nath A, Yeo LY, Sen AK (2021) Coalescence of droplets in a microwell driven by surface acoustic waves. Langmuir. https://doi.org/10.1021/acs.langmuir.0c03292
59. Mazutis L et al (2013) Single-cell analysis and sorting using droplet-based microfluidics. Nat Protoc 8:870–891
60. Huh D et al (2007) Gravity-driven microfluidic particle sorting device with hydrodynamic separation amplification. Anal Chem 79:1369–1376
61. Xi HD et al (2017) Active droplet sorting in microfluidics: a review. Lab Chip 17:751–771
62. Shang L, Cheng Y, Zhao Y (2017) Emerging droplet microfluidics. Chem Rev 117:7964–8040
63. Nooranidoost M, Haghshenas M, Muradoglu M, Kumar R (2019) Cell encapsulation modes in a flow-focusing microchannel: effects of shell fluid viscosity. Microfluid Nanofluid 23:1–10
64. Di Carlo D, Irimia D, Tompkins RG, Toner M (2007) Continuous inertial focusing, ordering, and separation of particles in microchannels. Proc Natl Acad Sci U S A 104:18892–18897
65. He M et al (2005) Selective encapsulation of single cells and subcellular organelles into picoliter- and femtoliter-volume droplets. Anal Chem 77:1539–1544
66. Zeng J et al (2012) Three-dimensional magnetic focusing of particles and cells in ferrofluid flow through a straight microchannel. J Micromech Microeng 22:105018
67. Kemna EWM et al (2012) High-yield cell ordering and deterministic cell-in-droplet encapsulation using Dean flow in a curved microchannel. Lab Chip 12:2881–2887
68. Kamalakshakurup G, Lee AP (2017) High-efficiency single cell encapsulation and size selective capture of cells in picoliter droplets based on hydrodynamic micro-vortices. Lab Chip 17:4324–4333
69. Gaikwad R, Sen AK (2021) An optomicrofluidic device for the detection and isolation of drop-encapsulated target cells in single-cell format. Analyst 146:95–108
70. Hemachandran E, Hoque SZ, Laurell T, Sen AK (2021) Reversible stream drop transition in a microfluidic coflow system via on demand exposure to acoustic standing waves. Phys Rev Lett 127:134501
71. Banerjee U, Jain SK, Sen AK (2021) Particle encapsulation in aqueous ferrofluid drops and sorting of particle-encapsulating drops from empty drops using a magnetic field. Soft Matter 17:6020–6028
72. Niculescu AG, Chircov C, Bîrcă AC, Grumezescu AM (2021) Fabrication and applications of microfluidic devices: a review. Int J Mol Sci 22:1–26
73. Choi K, Ng AHC, Fobel R, Wheeler AR (2012) Digital microfluidics. Annu Rev Anal Chem 5:413–440
74. Friend J, Yeo LY (2011) Microscale acoustofluidics: microfluidics driven via acoustics and ultrasonics. Rev Mod Phys. https://doi.org/10.1103/RevModPhys.83.647
75. Wenzel RN (1936) Resistance of solid surfaces to wetting by water. Ind Eng Chem 28:988–994
76. Brittain ST, Paul KE, Zhao X-M, Whitesides GM (1998) Soft lithography and microfabrication. Phys World 11:31–36
77. Luz GM, Leite ÁJ, Neto AI, Song W, Mano JF (2011) Wettable arrays onto superhydrophobic surfaces for bioactivity testing of inorganic nanoparticles. Mater Lett 65:296–299
78. Rahmawan Y, Xu L, Yang S (2013) Self-assembly of nanostructures towards transparent, superhydrophobic surfaces. J Mater Chem A 1:2955–2969
79. Xu T, Xu L-P, Zhang X, Wang S (2019) Bioinspired superwettable micropatterns for biosensing. Chem Soc Rev 48:3153–3165
80. Li J-F, Zhang Y-J, Ding S-Y, Panneerselvam R, Tian Z-Q (2017) Core–shell nanoparticle-enhanced Raman spectroscopy. Chem Rev 117:5002–5069
81. Song Y, Xu T, Xu L-P, Zhang X (2018) Superwettable nanodendritic gold substrates for direct miRNA SERS detection. Nanoscale 10:20990–20994
82. Hou J et al (2015) Hydrophilic–hydrophobic patterned molecularly imprinted photonic crystal sensors for high-sensitive colorimetric detection of tetracycline. Small 11:2738–2742

83. Connacher W, Orosco J, Friend J (2020) Droplet ejection at controlled angles via acoustofluidic jetting. Phys Rev Lett 125:184504
84. Sudeepthi A, Sen AK, Yeo L (2019) Aggregation of a dense suspension of particles in a microwell using surface acoustic wave microcentrifugation. Microfluid Nanofluid 23:76
85. Wiklund M, Green R, Ohlin M (2012) Acoustofluidics 14: Applications of acoustic streaming in microfluidic devices. Lab Chip. https://doi.org/10.1039/c2lc40203c
86. Aijian AP, Garrell RL (2015) Digital microfluidics for automated hanging drop cell spheroid culture. J Lab Autom 20:283–295
87. Nelson WC, Kim CJC (2012) Droplet actuation by electrowetting-on-dielectric (EWOD): a review. J Adhes Sci Technol 26:1747–1771
88. Mugele F, Baret J-C (2005) Electrowetting: from basics to applications. J Phys Condens Matter 17:R705–R774
89. Jones TB (2001) Liquid dielectrophoresis on the microscale. J Electrost 51–52:290–299
90. Kaler KVIS, Prakash R, Chugh D (2010) Liquid dielectrophoresis and surface microfluidics. Biomicrofluidics 4:022805
91. Sudeepthi A, Yeo L, Sen AK (2020) Cassie-Wenzel wetting transition on nanostructured superhydrophobic surfaces induced by surface acoustic waves. Appl Phys Lett 116:1–6
92. Zhang Y, Nguyen NT (2017) Magnetic digital microfluidics - a review. Lab Chip 17:994–1008
93. Mandal C, Banerjee U, Sen AK (2019) Transport of a sessile aqueous droplet over spikes of oil based ferrofluid in the presence of a magnetic field. Langmuir. https://doi.org/10.1021/acs.langmuir.9b00631
94. Sen P, Kim CJ (2009) Capillary spreading dynamics of electrowetted sessile droplets in air. Langmuir 25:4302–4305
95. Jones TB, Wang KL, Yao DJ (2004) Frequency-dependent electromechanics of aqueous liquids: electrowetting and dielectrophoresis. Langmuir 20:2813–2818
96. Li J, Kim CJ (2020) Current commercialization status of electrowetting-on-dielectric (EWOD) digital microfluidics. Lab Chip 20:1705–1712
97. Cho HM, Kim C-J (2003) Creating, transporting, cutting, and merging liquid droplets by electrowetting-based actuation for digital microfluidic circuits. J Microelectromech Syst 12:70–80
98. Pang L, Ding J, Liu X-X, Fan S-K (2019) Digital microfluidics for cell manipulation. TrAC Trends Anal Chem. https://doi.org/10.1016/j.trac.2019.06.008
99. Zhuang J, Yin J, Lv S, Wang B, Mu Y (2020) Advanced "lab-on-a-chip" to detect viruses – current challenges and future perspectives. Biosens Bioelectron 163:112291
100. Nasseri B et al (2018) Point-of-care microfluidic devices for pathogen detection. Biosens Bioelectron 117:112–128
101. Bansal S, Subramanian S (2021) A microfluidic acoustic metamaterial using electrowetting: enabling active broadband tunability. Adv Mater Technol 6:2100491
102. Pohl HA (1958) Some effects of nonuniform fields on dielectrics. J Appl Phys 29:1182–1188
103. Khoshmanesh K, Nahavandi S, Baratchi S, Mitchell A, Kalantar-zadeh K (2011) Dielectrophoretic platforms for bio-microfluidic systems. Biosens Bioelectron 26:1800–1814
104. Piao Y, Yu K, Jones TB, Wang W (2021) Electrical actuation of dielectric droplets by negative liquid dielectrophoresis. Electrophoresis 42(23):2490–2497. https://doi.org/10.1002/elps.202100093
105. Nampoothiri KN, Sen P (2021) Motion of generated dumbbell-shaped satellite droplets during liquid dielectrophoresis. J Micromech Microeng 31
106. Nampoothiri KN, Bobji MS, Sen P (2019) Generation of micron-sized droplet streams by high frequency electric fields. Int J Heat Mass Transf 145:118709
107. Nampoothiri KN, Srinivasan V, Bobji MS, Sen P (2017) A novel sub-picoliter monodispersed droplet generation device based on liquid dielectrophoresis. Proc IEEE Int Conf Micro Electro Mech Syst 2017:87–90. https://doi.org/10.1109/MEMSYS.2017.7863346

108. Nampoothiri KN, Seshasayee MS, Srinivasan V, Bobji MS, Sen P (2018) Direct heating of aqueous droplets using high frequency voltage signals on an EWOD platform. Sensors Actuators B Chem 273:862–872
109. Nampoothiri KN, Bobji MS, Sen P (2020) De-icing device with self-adjusting power consumption and ice sensing capabilities. J Microelectromech Syst 29:562–570
110. Frozanpoor I et al (2021) Programmable droplet actuating platform using liquid dielectrophoresis. J Micromech Microeng 31:055014
111. Settnes M, Bruus H (2012) Forces acting on a small particle in an acoustical field in a viscous fluid. Phys Rev E Stat Nonlinear Soft Matter Phys 85:1–12
112. Bruus H et al (2011) Forthcoming lab on a chip tutorial series on acoustofluidics: acoustofluidics - exploiting ultrasonic standing wave forces and acoustic streaming in microfluidic systems for cell and particle manipulation. Lab Chip 11:3579–3580
113. Ding X et al (2013) Surface acoustic wave microfluidics. Lab Chip 13:3626
114. Rasouli R, Tabrizian M (2021) Rapid formation of multicellular spheroids in boundary-driven acoustic microstreams. Small 17:1–12
115. Dolatmoradi A, Mirtaheri E, El-Zahab B (2017) Thermo-acoustofluidic separation of vesicles based on cholesterol content. Lab Chip 17:1332–1339
116. Dow P, Kotz K, Gruszka S, Holder J, Fiering J (2018) Acoustic separation in plastic microfluidics for rapid detection of bacteria in blood using engineered bacteriophage. Lab Chip 18:923–932
117. Ohlsson P, Petersson K, Augustsson P, Laurell T (2018) Acoustic impedance matched buffers enable separation of bacteria from blood cells at high cell concentrations. Sci Rep 8:1–11
118. Lenshof A, Magnusson C, Laurell T (2012) Acoustofluidics 8: Applications of acoustophoresis in continuous flow microsystems. Lab Chip 12:1210–1223
119. Antfolk M, Magnusson C, Augustsson P, Lilja H, Laurell T (2015) Acoustofluidic, label-free separation and simultaneous concentration of rare tumor cells from white blood cells. Anal Chem 87:9322–9328
120. Grenvall C, Magnusson C, Lilja H, Laurell T (2015) Concurrent isolation of lymphocytes and granulocytes using prefocused free flow acoustophoresis. Anal Chem 87:5596–5604
121. Johnson DA, Feke DL (1995) Methodology for fractionating suspended particles using ultrasonic standing wave and divided flow fields. Sep Technol 5:251–258
122. Chen Y et al (2016) High-throughput acoustic separation of platelets from whole blood. Lab Chip 16:3466–3472
123. Antfolk M, Laurell T (2017) Continuous flow microfluidic separation and processing of rare cells and bioparticles found in blood – a review. Anal Chim Acta 965:9–35
124. Petersson F, Åberg L, Swärd-Nilsson AM, Laurell T (2007) Free flow acoustophoresis: microfluidic-based mode of particle and cell separation. Anal Chem 79:5117–5123
125. Karthick S, Sen AK (2018) Improved understanding of acoustophoresis and development of an acoustofluidic device for blood plasma separation. Phys Rev Appl 10:1
126. Karthick S, Pradeep PN, Kanchana P, Sen AK (2018) Acoustic impedance-based size-independent isolation of circulating tumour cells from blood using acoustophoresis. Lab Chip 18:3802–3813
127. Piyasena ME et al (2012) Multinode acoustic focusing for parallel flow cytometry. Anal Chem 84:1831–1839
128. Goddard G, Martin JC, Graves SW, Kaduchak G (2006) Ultrasonic particle-concentration for sheathless focusing of particles for analysis in a flow cytometer. Cytometry A. https://doi.org/10.1002/cyto.a.20205
129. Kalb DM et al (2017) Line-focused optical excitation of parallel acoustic focused sample streams for high volumetric and analytical rate flow cytometry. Anal Chem. https://doi.org/10.1021/acs.analchem.7b02319
130. Jakobsson O, Grenvall C, Nordin M, Evander M, Laurell T (2014) Acoustic actuated fluorescence activated sorting of microparticles. Lab Chip 14:1943–1950

131. Jakobsson O et al (2015) Thousand-fold volumetric concentration of live cells with a recirculating acoustofluidic device. Anal Chem 87:8497–8502
132. Wiklund M, Radel S, Hawkes JJ (2013) Acoustofluidics 21: Ultrasound-enhanced immunoassays and particle sensors. Lab Chip 13:25–39
133. Reboud J et al (2012) Shaping acoustic fields as a toolset for microfluidic manipulations in diagnostic technologies. Proc Natl Acad Sci U S A 109:15162–15167
134. Gracioso Martins AM et al (2014) Toward complete miniaturisation of flow injection analysis systems: microfluidic enhancement of chemiluminescent detection. Anal Chem 86:10812–10819
135. Destgeer G et al (2014) Adjustable, rapidly switching microfluidic gradient generation using focused travelling surface acoustic waves. Appl Phys Lett 104:10–15
136. Cecchini M, Girardo S, Pisignano D, Cingolani R, Beltram F (2008) Acoustic-counterflow microfluidics by surface acoustic waves. Appl Phys Lett 92:2006–2009
137. Zhang SP et al (2018) Digital acoustofluidics enables contactless and programmable liquid handling. Nat Commun 9:1–11
138. Bourquin Y, Reboud J, Wilson R, Zhang Y, Cooper JM (2011) Integrated immunoassay using tuneable surface acoustic waves and lensfree detection. Lab Chip 11:2725–2730
139. Collins DJ, Ma Z, Ai Y (2016) Highly localized acoustic streaming and size-selective submicrometer particle concentration using high frequency microscale focused acoustic fields. Anal Chem 88:5513–5522
140. Bourquin Y et al (2014) Rare-cell enrichment by a rapid, label-free, ultrasonic isopycnic technique for medical diagnostics. Angew Chem Int Ed 53:5587–5590
141. Destgeer G et al (2016) Acoustofluidic particle manipulation inside a sessile droplet: four distinct regimes of particle concentration. Lab Chip 16:660–667
142. Lee K, Shao H, Weissleder R, Lee H, Al LEEET (2015) Acoustic purification of extracellular microvesicles. ACS Nano 9(3):2321–2327
143. Ding X et al (2012) Standing surface acoustic wave (SSAW) based multichannel cell sorting. Lab Chip 12:4228–4231
144. Chen Y et al (2014) Continuous enrichment of low-abundance cell samples using standing surface acoustic waves (SSAW). Lab Chip 14:924–930
145. Nam J, Lim H, Kim D, Shin S (2011) Separation of platelets from whole blood using standing surface acoustic waves in a microchannel. Lab Chip 11:3361–3364
146. Shi J, Mao X, Ahmed D, Colletti A, Huang TJ (2008) Focusing microparticles in a microfluidic channel with standing surface acoustic waves (SSAW). Lab Chip 8:221–223
147. Ren L et al (2015) A high-throughput acoustic cell sorter. Lab Chip 15:3870–3879
148. Shi J, Huang H, Stratton Z, Huang Y, Huang TJ (2009) Continuous particle separation in a microfluidic channel via standing surface acoustic waves (SSAW). Lab Chip 9:3354–3359
149. Ren L et al (2018) Standing surface acoustic wave (SSAW)-based fluorescence-activated cell sorter. Small 14:1–8
150. Chen Y et al (2014) Standing surface acoustic wave (SSAW)-based microfluidic cytometer. Lab Chip 14:916–923
151. Ai Y, Sanders CK, Marrone BL (2013) Separation of Escherichia coli bacteria from peripheral blood mononuclear cells using standing surface acoustic waves. Anal Chem 85:9126–9134
152. Wang Z et al (2020) Erratum: Isolation of exosomes from whole blood by integrating acoustics and microfluidics (Proceedings of the National Academy of Sciences of the United States of America (2017) 114 (10584–10589) DOI: 10.1073/pnas.1709210114). Proc Natl Acad Sci U S A 117:28525
153. Ding X et al (2014) Cell separation using tilted-angle standing surface acoustic waves. Proc Natl Acad Sci U S A 111:12992–12997
154. Dao M et al (2015) Acoustic separation of circulating tumor cells. Proc Natl Acad Sci U S A 112:4970–4975
155. Wu M et al (2018) Circulating tumor cell phenotyping via high-throughput acoustic separation. Small 14:1–10

156. Franke T, Braunmüller S, Schmid L, Wixforth A, Weitz DA (2010) Surface acoustic wave actuated cell sorting (SAWACS). Lab Chip 10:789–794
157. Ma Z, Collins DJ, Ai Y (2016) Detachable acoustofluidic system for particle separation via a traveling surface acoustic wave. Anal Chem 88:5316–5323
158. Ung WL et al (2017) Enhanced surface acoustic wave cell sorting by 3D microfluidic-chip design. Lab Chip 17:4059–4069
159. Destgeer G, Ha BH, Jung JH, Sung HJ (2014) Submicron separation of microspheres via travelling surface acoustic waves. Lab Chip 14:4665–4672
160. Ahmed D et al (2016) Rotational manipulation of single cells and organisms using acoustic waves. Nat Commun 7:11085
161. Lee AP, Patel MV, Tovar AR, Okabe Y (2010) Microfluidic air-liquid cavity acoustic transducers for on-chip integration of sample preparation and sample detection. J Assoc Lab Autom 15:449–454
162. Rasouli MR, Tabrizian M (2019) An ultra-rapid acoustic micromixer for synthesis of organic nanoparticles. Lab Chip 19:3316–3325
163. Doinikov AA, Gerlt MS, Dual J (2020) Acoustic radiation forces produced by sharp-edge structures in microfluidic systems. Phys Rev Lett 124:154501
164. Huang PH et al (2014) A reliable and programmable acoustofluidic pump powered by oscillating sharp-edge structures. Lab Chip 14:4319–4323
165. Huang PH et al (2013) An acoustofluidic micromixer based on oscillating sidewall sharp-edges. Lab Chip 13:3847–3852
166. Huang PH et al (2015) An acoustofluidic sputum liquefier. Lab Chip 15:3125–3131
167. Wang Z et al (2019) Cell lysis: via acoustically oscillating sharp edges. Lab Chip 19:4021–4032
168. Leibacher I, Hahn P, Dual J (2015) Acoustophoretic cell and particle trapping on microfluidic sharp edges. Microfluid Nanofluid 19:923–933
169. Shanti A, Teo J, Stefanini C (2018) In vitro immune organs-on-chip for drug development: a review. Pharmaceutics 10:278
170. McKim J Jr (2010) Building a tiered approach to in vitro predictive toxicity screening: a focus on assays with in vivo relevance. Comb Chem High Throughput Screen 13:188–206
171. Prantil-Baun R et al (2018) Physiologically based pharmacokinetic and pharmacodynamic analysis enabled by microfluidically linked organs-on-chips. Annu Rev Pharmacol Toxicol 58:37–64
172. Sager JE, Yu J, Ragueneau-Majlessi I, Isoherranen N (2015) Physiologically based pharmacokinetic (PBPK) modeling and simulation approaches: a systematic review of published models, applications, and model verification. Drug Metab Dispos 43:1823–1837
173. Rowland M, Peck C, Tucker G (2011) Physiologically-based pharmacokinetics in drug development and regulatory science. Annu Rev Pharmacol Toxicol 51:45–73
174. Kankala RK, Wang S-B, Chen A-Z (2019) Microengineered organ-on-a-chip platforms towards personalized medicine. Curr Pharm Des 24:5354–5366
175. Esch EW, Bahinski A, Huh D (2015) Organs-on-chips at the frontiers of drug discovery. Nat Rev Drug Discov 14:248–260
176. Huh D, Hamilton GA, Ingber DE (2011) From 3D cell culture to organs-on-chips. Trends Cell Biol 21:745–754
177. Huh D et al (2013) Microfabrication of human organs-on-chips. Nat Protoc 8:2135–2157
178. Yum K, Hong SG, Healy KE, Lee LP (2014) Physiologically relevant organs on chips. Biotechnol J 9:16–27
179. Zhang YS et al (2017) Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proc Natl Acad Sci U S A 114:E2293–E2302
180. Perestrelo AR, Águas ACP, Rainer A, Forte G (2015) Microfluidic organ/body-on-a-chip devices at the convergence of biology and microengineering. Sensors (Switzerland) 15:31142–31170

181. Huh D et al (2010) Reconstituting organ-level lung functions on a chip. Science 328:1662–1668
182. Inamdar NK, Borenstein JT (2011) Microfluidic cell culture models for tissue engineering. Curr Opin Biotechnol 22:681–689
183. Khetani SR, Bhatia SN (2008) Microscale culture of human liver cells for drug development. Nat Biotechnol 26:120–126
184. Bhatia SN, Ingber DE (2014) Microfluidic organs-on-chips. Nat Biotechnol 32:760–772
185. Mittal R et al (2019) Organ-on-chip models: implications in drug discovery and clinical applications. J Cell Physiol 234:8352–8380
186. Arrigoni C, Gilardi M, Bersini S, Candrian C, Moretti M (2017) Bioprinting and organ-on-chip applications towards personalized medicine for bone diseases. Stem Cell Rev Rep 13: 407–417
187. Skardal A et al (2017) Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. Sci Rep 7:1–16
188. Lee SH, Sung JH (2018) Organ-on-a-chip technology for reproducing multiorgan physiology. Adv Healthc Mater 7:1–17
189. Ronaldson-Bouchard K, Vunjak-Novakovic G (2018) Organs-on-a-chip: a fast track for engineered human tissues in drug development. Cell Stem Cell 22:310–324
190. Zamprogno P et al (2021) Second-generation lung-on-a-chip with an array of stretchable alveoli made with a biological membrane. Commun Biol 4:1–10
191. Zhang M et al (2021) Biomimetic human disease model of SARS-CoV-2-induced lung injury and immune responses on organ chip system. Adv Sci 8:1–14
192. Singh AV et al (2021) Advances in smoking related in vitro inhalation toxicology: a perspective case of challenges and opportunities from progresses in lung-on-chip technologies. Chem Res Toxicol. https://doi.org/10.1021/acs.chemrestox.1c00219
193. Huang D et al (2021) Reversed-engineered human alveolar lung-on-a-chip model. Proc Natl Acad Sci U S A 118:1–10
194. Benam KH et al (2015) Engineered in vitro disease models. Annu Rev Pathol Mech Dis 10: 195–262
195. Jin Y et al (2018) Three-dimensional brain-like microenvironments facilitate the direct reprogramming of fibroblasts into therapeutic neurons. Nat Biomed Eng 2:522–539
196. Estlack Z, Bennet D, Reid T, Kim J (2017) Microengineered biomimetic ocular models for ophthalmological drug development. Lab Chip 17:1539–1551
197. Ma C, Peng Y, Li H, Chen W (2021) Organ-on-a-chip: a new paradigm for drug development. Trends Pharmacol Sci 42:119–133
198. Wu Q et al (2020) Organ-on-a-chip: recent breakthroughs and future prospects. Biomed Eng Online 19:1–19
199. Yu F, Hunziker W, Choudhury D (2019) Engineering microfluidic organoid-on-a-chip platforms. Micromachines 10:1–12
200. Chinen AB et al (2017) Nanoparticle probes for the detection of cancer biomarkers. Cells Tissues Fluoresc 115:10530–10574
201. Shi W et al (2017) Magnetic particles assisted capture and release of rare circulating tumor cells using wavy-herringbone structured microfluidic devices. Lab Chip 17:3291–3299
202. Viganò P et al (2001) Development and characterization of microfluidic devices and systems for magnetic bead-based biochemical detection. Biomed Microdevices 3:191–200
203. Binkley MM, Cui M, Berezin MY, Meacham JM (2020) Antibody conjugate assembly on ultrasound-confined microcarrier particles. ACS Biomater Sci Eng 6:6108–6116
204. Nagrath S et al (2007) Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature 450:1235–1239
205. Austin Suthanthiraraj PP, Sen AK (2019) Localized surface plasmon resonance (LSPR) biosensor based on thermally annealed silver nanostructures with on-chip blood-plasma separation for the detection of dengue non-structural protein NS1 antigen. Biosens Bioelectron 132:38–46

206. Khan NI, Song E (2020) Lab-on-a-chip systems for aptamer-based biosensing. Micromachines 11:220
207. Chen Y, Pulikkathodi K, Ma Y, Wang Y, Lee G (2019) Lab on a Chip transistors for enumeration of circulating tumor. Lab Chip. https://doi.org/10.1039/c8lc01072b
208. Song S, Wang L, Li J, Fan C, Zhao J (2008) Aptamer-based biosensors. Trends Anal Chem 27: 108–117
209. Campaña AL et al (2019) Enzyme-based electrochemical biosensors for microfluidic platforms to detect pharmaceutical residues in wastewater. Biosensors 9:41
210. Hernández-Ibáñez N et al (2016) Electrochemical lactate biosensor based upon chitosan/carbon nanotubes modified screen-printed graphite electrodes for the determination of lactate in embryonic cell cultures. Biosens Bioelectron 77:1168–1174
211. Brahim S, Narinesingh D, Guiseppi-Elie A (2002) Polypyrrole-hydrogel composites for the construction of clinically important biosensors. Biosens Bioelectron 17:53–59
212. Maji SK, Sreejith S, Mandal AK, Ma X, Zhao Y (2014) Immobilizing gold nanoparticles in mesoporous silica covered reduced graphene oxide: a hybrid material for cancer cell detection through hydrogen peroxide sensing. ACS Appl Mater Interfaces 6:13648–13656
213. Jiang S et al (2013) Real-time electrical detection of nitric oxide in biological systems with sub-nanomolar sensitivity. Nat Commun 4:1–7
214. Karunya R et al (2019) Rapid measurement of hydrogen sulphide in human blood plasma using a microfluidic method. Sci Rep 9:1–11
215. Gaikwad R, Thangaraj PR, Sen AK (2021) Direct and rapid measurement of hydrogen peroxide in human blood using a microfluidic device. Sci Rep 11:1–10
216. Del Ben F et al (2016) A method for detecting circulating tumor cells based on the measurement of single-cell metabolism in droplet-based microfluidics. Angew Chem Int Ed Engl. https://doi.org/10.1002/anie.201602328
217. Liberti MV, Locasale JW (2016) The Warburg effect: how does it benefit cancer cells? (vol 41, pg 211, 2016). Trends Biochem Sci 41:287
218. Balcells M et al (2002) Reactive polymer coatings: a platform for patterning proteins and mammalian cells onto a broad range of materials. Langmuir 9:3632–3638
219. Khademhosseini A, Yeh J, Jon S, Eng G, Suh KY (2004) Molded polyethylene glycol microstructures for capturing cells within microfluidic channels. Lab Chip 4:425–430
220. Vijayasai AP et al (2010) Haptic controlled three-axis MEMS gripper system. Rev Sci Instrum 81:105114
221. Tang Y et al (2014) Microfluidic device with integrated microfilter of conical-shaped holes for high efficiency and high purity capture of circulating tumor cells. Sci Rep. https://doi.org/10.1038/srep06052
222. Di Carlo D, Aghdam N, Lee LP (2006) Single-cell enzyme concentrations, kinetics, and inhibition analysis using high-density hydrodynamic cell isolation arrays. Anal Chem 78: 4925–4930
223. Kim H, Lee S, Lee JH, Kim J (2015) Integration of a microfluidic chip with a size-based cell bandpass filter for reliable isolation of single cells. Lab Chip 15:4128–4132
224. Ahmed MG et al (2017) Isolation, detection, and antigen-based profiling of circulating tumor cells using a size-dictated immunocapture chip. Angew Chem Int Ed Engl 129:10821–10825
225. Teh SY, Lin R, Hung LH, Lee AP (2008) Droplet microfluidics. Lab Chip 8:198–220. https://doi.org/10.1039/B715524G
226. Fu AY, Spence C, Scherer A, Arnold FH, Quake SR (1999) A microfabricated fluorescence-activated cell sorter. Nat Biotechnol 17:1109–1111
227. Wyss HM, Blair DL, Morris JF, Stone HA, Weitz DA (2006) Mechanism for clogging of microchannels. Phys Rev E Stat Nonlinear Soft Matter Phys 74:1–4
228. Park J et al (2006) Asymmetric nozzle structure for particles converging into a highly confined region. Curr Appl Phys 6:992–995
229. Marella SV, Udaykumar HS (2004) Computational analysis of the deformability of leukocytes modeled with viscous and elastic structural components. Phys Fluids 16:244–264

230. Huh D et al (2002) Use of air-liquid two-phase flow in hydrophobic microfluidic channels for disposable flow cytometers. Biomed Microdevices 4:141–149
231. Choi S, Song S, Choi C, Park JK (2008) Sheathless focusing of microbeads and blood cells based on hydrophoresis. Small 4:634–641
232. Sajeesh P, Manasi S, Doble M, Sen AK (2015) A microfluidic device with focusing and spacing control for resistance-based sorting of droplets and cells. Lab Chip 15:3738–3748
233. Alazzam A, Mathew B, Alhammadi F (2017) Novel microfluidic device for the continuous separation of cancer cells using dielectrophoresis. J Sep Sci 40:1193–1200
234. Wang L et al (2009) Dual frequency dielectrophoresis with interdigitated sidewall electrodes for microfluidic flow-through separation of beads and cells. Electrophoresis 30:782–791
235. Wang X et al (2011) Enhanced cell sorting and manipulation with combined optical tweezer and microfluidic chip technologies. Lab Chip 11:3656–3662
236. Palmer RMJ, Ferrige AG, Moncada S (1987) Optical trapping and manipulation of single cells using infrared laser beam. Nature 327:524–526
237. Zhang Z, Kimkes TEP, Heinemann M (2019) Manipulating rod-shaped bacteria with optical tweezers. Sci Rep 9:1–9
238. Chiu TK et al (2016) Application of optically-induced-dielectrophoresis in microfluidic system for purification of circulating tumour cells for gene expression analysis-cancer cell line model. Sci Rep 6:1–14
239. Robert D et al (2011) Cell sorting by endocytotic capacity in a microfluidic magnetophoresis device. Lab Chip 11:1902–1910
240. Shen F, Hwang H, Hahn YK, Park JK (2012) Label-free cell separation using a tunable magnetophoretic repulsion force. Anal Chem 84:3075–3081
241. Rodríguez-Villarreal AI et al (2011) Flow focussing of particles and cells based on their intrinsic properties using a simple diamagnetic repulsion setup. Lab Chip 11:1240–1248
242. Zeng J, Chen C, Vedantam P, Tzeng TR, Xuan X (2013) Magnetic concentration of particles and cells in ferrofluid flow through a straight microchannel using attracting magnets. Microfluid Nanofluid 15:49–55

Microfluidics-Based Organ-on-a-Chip for Cell Biology Studies

3

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Abstract

Understanding of cellular behaviour and functions is necessary to model various disease conditions and further to develop novel therapeutics. However, most of the in vitro test relies on the cells grown on Petri plates, which lacks the media perfusion, cell–matrix interaction or physiological forces. Therefore, significant improvement has been made to develop more physiologically relevant cell/organ models. Microfluidics is the latest technology that is characterised by fluid manipulation to develop an artificial cell environment and to mimic the complex nature of tissues. Utilising the potential of microfluidics in biomedical researches has revolutionised conventional cell culture methods and animal testing. An emerging class of microfluidic devices tries to replicate functions of major organs in vivo on certain chips called organ-on-a-chip (OOC). Such organ-on-a-chip development integrates several aspects of microfluidic technology to form an in vitro device model. The in vitro device models will help in mimicking the complex nature of native tissues, which will in turn help researchers to deeply study the normal and diseased conditions of several organs. The new microfluidic device called organ-on-a-chip includes lung-on-a-chip, kidney-on-a-chip, cancer-on-a-chip, heart-on-a-chip, blood-vessel-on-chip, etc. Utilising such chips, several assays can be performed to determine the toxicity of several compounds, cellular response to various physiological forces, cell signalling, etc. Hence, this chapter explains the organ-on-a-chip technology and its application in cell biology experiments.

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3.1 Introduction

The advances in cell biology techniques have increased the understanding of the progression and pathophysiology of human diseases and demonstrated a significant role in the drug-development pipeline. Nevertheless, the current preclinical evaluation methods are often associated with many challenges. The lion's share of the cost spent during the drug molecule development is associated with the preclinical studies. Similarly, most of the drug candidate molecules that qualify the preclinical studies would not reach the market due to their unexpected toxicity and the discrepancy in their expected efficacy. The absence of an accurate model that mimics the disease condition and the inadequate understanding of cellular and molecular mechanisms of the pathological condition and drug action can hinder the drug development process. Drug-led molecule screening and efficacy studies are conventionally performed in cells grown on Petri plates. These cells cannot accurately recapitulate the actual in vivo condition. In the actual scenario, the cells are under the influence of many physiological forces, chemical gradients, etc., which is absent in the cells grown on Petri plates. Therefore, the model system that recapitulates the actual cellular condition and cellular microenvironment is required to speed up the drug development process.

Cell biology is a field of scientific research that deals with studying cells, the basic functional unit of a living system. The cells consist of cytoplasm that contains nucleus and organelles enclosed in a plasma membrane, which is made up of lipid bilayer. Therefore, a cell biologist studies the regulation of various cellular processes, cellular communication, cellular microenvironments, cellular organisation and composition. The difference in the behaviour of different cell types is also essential. Cells contain various organelles, and these organelles closely work together. The biosynthesis of proteins, intracellular movement and, finally, the secretion from the cell are also studied in cell biology. The biosynthesis of proteins is usually carried out by ribosomes present in the cell's cytoplasm. Some newly synthesised proteins translocate to the endoplasmic reticulum and are later processed in the Golgi apparatus. The processed protein further moves to the subcellular regions of the cellular membrane, and some of the proteins can secrete from the cell. Cell biology also focuses on the various transport mechanisms such as active and passive transport or the simple diffusion present in the cell, cellular adhesion and organisation, cell division, various cell signalling pathways, cellular metabolism, etc.

The cellular microenvironment is a crucial factor that determines many cellular functions. Many biochemical and biophysical cues are present and synergistically play a crucial role in establishing the cellular microenvironment. Reconstituting the in-vivo-like microenvironment is, therefore, necessary to obtain physiologically

relevant results from the studies. Recent improvements in tissue engineering and cell biology led to the development of three-dimensional (3D) cell culture techniques, which were able to mimic certain aspects of the cellular microenvironment by employing the extracellular matrix (ECM)-like materials. Various hydrogel materials have been developed to mimic the complex natural cell microenvironment. These hydrogels can be classified as synthetically or naturally derived. Naturally derived hydrogels are prepared from the polymer of natural sources such as collagen, fibrin, and gelatin. The main advantages of naturally derived hydrogels include biocompatibility and inherent cell adhesion ligands; however, they often possess batch-to-batch variation. The synthetically derived hydrogels are prepared from the crosslinking of synthetic polymers such as polyvinyl alcohol (PVA), poly(ethylene glycol) (PEG), and polyacrylamide (PAA). The 3D cell culture studies can recapitulate the structural aspect of the cellular microenvironment; however, they often lack the media perfusion and continuous removal of waste.

Incorporating microfabrication technologies and microfluidics into cell biology greatly enhanced the successful mimicking of the cellular microenvironments and recapitulating the organ-level functions. Microfabrication techniques are derived initially from the microelectronics industry.

3.2 Organ-on-a-Chip

Organ-on-a-chip (OOC) systems are microscale devices used to mimic the organ-level functions in vitro [1]. The organ-on-a-chip system can provide a physiologically relevant model of organ system by incorporating various biological and mechanical cues affecting the cellular microenvironment [2]. The organ-on-a-chip system gained considerable momentum in recent years due to its ability to develop disease models. Most of the organs-on-a-chip developed so far focused on the recapitulation of single organ function. However, the development of technology helped to develop multi-organ-on-a-chip (MOC) models either in the same chip or different chips connected to each other via fluidic connections [3]. These organ-on-a-chip models are not only helpful in the assessment of drug molecule but also provide insight towards the organ-level interactions such as the metabolic coupling present in the blood-brain barriers and neurons. There are many advantages of organ-on-a-chip systems, including incorporating microelectrodes to measure various parameters associated with cellular response and growth directly.

Organ-on-a-chip has the upper hand over the conventional in vitro culture or the three-dimensional (3D) culture due to properly mimicking the tissues perfused by blood vessels. The fluidic connections present in the organ-on-a-chip device can recapitulate the vasculatures present in the tissue in vitro. Hence, it can model the spatiotemporal gradient of nutrients, the hypoxia condition in the tumour microenvironments, infiltrations of immune cells and so on [4]. The mechanical forces such as the peristalsis movement in the gastrointestinal tract, cyclic stretching in the alveolar epithelial cells, fluid shear stress in renal epithelial cells, etc. can generate in the organ-on-a-chip system. The continuous flow through the fluidic

connections of organ-on-a-chip enables the expansion of progenitor cells such as endothelial cells, which is vital in developing various organoid models. Continuous screening of various biological parameters is also possible in organ-on-a-chip models. Organs-on-a-chip are microfluidic devices that utilise media and other reagent in a very small quantity such as in microscale level. Coculture of various cells is also possible in organs-on-a-chip.

3.3 Organ-on-a-Chip for Cell Biology and Understanding Disease Progression

Pharmaceutical drug testing methods traditionally use animal models to evaluate the pharmacological and toxicological effects of a new drug entity. However, these methods lack the ability to directly reflect the physiological responses. A more accurate reflection of the pharmacological and toxicological responses can be captured by the lung-on-a-chip model compared to the conventional in vitro models. Lung-on-a-chip is a micro-engineered miniature cell culture device that reproduces the 3D microenvironment and microarchitecture, breathing movements, and primary physiological functions of the human lung. The identification of human lungs physiology, toxicological studies, drug screening and the disease aetiology can be the potential application of the lung-on-a-chip models. The traditional two-dimensional (2D) cell culture methods and animal studies are nearly to decrease in the near future if the organ-on-a-chip technology is widely accepted and utilised. Lithography-based microfabrication techniques, thermoplastic technique and 3D bioprinting are the techniques used to fabricate lung-on-a-chip models. The evaluation of pathophysiological mechanisms of different lung diseases becomes effective by utilising successfully fabricated lung disease models. Lung cancer, pulmonary oedema, pulmonary thrombosis, asthma and chronic obstructive pulmonary disease (COPD), and cystic fibrosis are some of the disease models elaborately studied using the lung-on-a-chip model.

The liver is the largest organ in the body with diverse metabolic functions, and plays a crucial role in maintaining the body's physiological functions. Chronic liver diseases or viral infections can alter the regenerative and recuperative properties of the liver and lead to permanent damage of the liver and loss of its functions. The studies of the mechanisms of liver diseases are conventionally carried out in in vitro models. The limitations of these studies have led to the rise of liver-on-a-chip microsystems that can contribute to the effective studies of the liver microenvironments. The liver-on-a-chip systems can be broadened to recapitulate the 3D culture methods, which are essential for liver physiology and to replicate liver functions.

The organ-on-a-chip platform can also be made by using cells from patients, and it can aid in developing personalised medicine. With recent developments in stem cell technology and tissue engineering, it is possible to develop organ-on-a-chip to tackle rare diseases. Such organ-on-a-chip is beneficial in predicting the drug response invulnerable populations and in developing novel therapeutics.

Most of the organs-on-a-chip are focusing on single organ equivalent. However, different organs-on-a-chip can connect to form a multi-organ-on-a-chip (MOC) model. MOC models are more complex than the single-organ-on-a-chip models since they require proper scaling of organ size, fluid flow rate and cell-seeding density. Integrating more organ equivalents within the same chip offers a more systemic level of human biology, referred to as 'human-on-a-chip' technology. This technology can provide a better understanding of dose responses and mechanisms of toxicity of drugs and an excellent replacement to animal models for preclinical toxicity studies. Human-on-a-chip is relatively a new area of cutting-edge research wherein researchers are working towards building a multi-channel 3D microfluidic cell culture system that compartmentalises microenvironments in which 3D cellular aggregates are cultured to mimic multiple organs in the body. The temporal control over the chemical simulation and the reduction of temporal lag in extracellular response towards the molecules, which is transported across the cellular membrane by reducing the ratio of extracellular to intracellular fluid volumes, promote a paradigm shift in the drug development process.

For example, the multi-organ model of the first-pass metabolism developed by Herland and co-workers from Wyss Institute, USA, utilises individual gut, liver and kidney chips connected through the vascular channel to predict pharmacokinetics/pharmacodynamics (PK/PD) of nicotine and cisplatin. For modelling nicotine, they added the nicotine to the lumen side of the intestine chip (gut chip), where it mimics the oral route of exposure and uptake of nicotine. Further, it crosses the intestinal wall and reaches the liver through the vascular channel. Nicotine metabolises in the liver, is transported to the kidney and is eliminated from the body. The modular system developed from the Wyss Institute allows the quantitative prediction and tissue-specific distribution of chemicals.

Human-on-a-chip connecting different organs can be a perfect PK/PD model for studying the responses of different organs to various drug molecules. Nevertheless, an ideal PK/PD model can lead to the improvement in existing treatment strategies for various diseases as well as the invention of new drug molecules. But the fabrication of an MOC by integrating different organs is not an easy process. The main challenges include organ scaling, the nurturing of different cell types in a single device and the availability of universal media for all cell types. Adding to that, the technical challenges like ideal multi-organ design connecting different organs, sterility of the device, etc. enhance the difficulty of fabricating MOCs. Despite all of these challenges, the multi-organ-on-a-chip can be a novel model with applications in next-generation healthcare (Table 3.1).

3.4 Liver-on-a-Chip for Cell Biology Studies

Liver-on-a-chip is one of the critically studied organ-on-a-chip models due to the importance of the liver in the detoxification and metabolism of toxicants and drugs [27]. Various liver-on-a-chip devices were developed to study the drug-induced effects on liver functions, disease modelling and the physico-chemical

Table 3.1 Examples of various organ-on-a-chip models developed and their objectives

Organ-on-a-chip model	Objectives	Reference
Lung-on-a-chip	Modelling of nanotoxicity	[5]
	To assess the effect of liquid plugs and gas flow in alveoli disease progression	[6]
	To mimic the breathing pattern and to model pulmonary oedema	[7]
	To assess the effect of cyclic stretch and fluid mechanical stress on the morphological change	[8]
	To assess the effect of environmental toxicant	[9]
	Modelling of absorption of aerosolised drugs	[10]
Blood-brain-barrier-on-a-chip	To develop the dynamic cerebrovascular environment	[11]
Heart-on-a-chip	To study the effect of epinephrine on the contractility of cardiomyocytes	[12]
	To study the effect of isoproterenol on the contractility of cardiac muscle	[13]
	Modelling of hypertrophic cardiomyopathy	[14]
	Modelling of myocardial injury by the induction of hypoxia	[15]
Kidney-on-a-chip	To model nephron function	[16]
	Modelling of cisplatin nephron toxicity	[17]
Spleen-on-a-chip	Modelling of the microenvironment of the spleen and its ability to distinguish red blood cells based on their mechanical properties	[18]
Liver-on-a-chip	To assess the effect of perfusion on the albumin synthesis and urea production	[19]
	To study the role of endothelial cells on the activities of hepatocytes	[20]
	To assess the effect of shear stress on hepatocytes	[21]
	To assess the effect of hepatic stellate cells on the formation of tight cell contacts and spheroids	[22]
	Modelling of liver sinusoids and to check the in vitro toxicity of rifampicin, acetaminophen and isoniazid	[23]
Brain-on-a-chip	To assess the effect of fluid flow on the neural network and differentiation	[24]
	To assess the effect of topological factors on the outgrowth of neurons	[25]
Womb-on-a-chip	Infertility studies	[26]

microenvironment. These liver-on-a-chip systems promote the long-term culture of functional liver constructs compared to the conventional systems. Different liver cellular responses such as variation in the secretion of biomarkers like albumin, transferring and alpha-1 antitrypsin can be monitored by the platform [27]. The combination of bioprinting and microfabrication technologies facilitates the designing and fabrication of accurately controlled three-dimensional microarchitectures [28, 29]. Compared to the monolayer cell culture, three-dimensional cell culture

models of liver cells better mimic the in vivo cell function with enhanced albumin production, and oxygen gradient in the liver lobule [30]. The continuous media perfusion through the microfluidic technique offers the supply of nutrients and oxygen and the removal of waste. Moreover, the spheroid cultures of hepatic constructs can enhance cells' functionalities, including homotypic cell–cell interaction [31–33], giving a better response to the drug candidate molecules [34].

The functional liver models require proper cell patterning similar to the in vivo model. The control over the spatial arrangement of various cells such as hepatocytes and parenchymal cells is critical in the recapitulation of the functional hepatic lobule. Various micro-patterning techniques such as those using photosensitive materials, thermoresponsive materials, dielectrophoresis and printing techniques have been developed to mimic the in vivo condition [35]. Micromachining methods have been demonstrated as a suitable tool for developing liver ultrastructures such as the liver sinusoids [36], bile ducts [37] and liver vasculatures [38]. Fabricating liver-specific ultrastructure is essential in mimicking the mass transfer occurring in the liver, such as the nutrients absorption, gaseous exchange and excretion of waste, and thereby enables long-term culture. Generation of endothelial-like barrier, the ultrastructure of vasculature using two-dimensional templates, and concave microwells on the microfluidic devices has been reported to mimic the liver microstructures [39].

The liver-on-a-chip devices have been employed for various cell biology studies such as cellular interaction studies, cell signalling studies and for studies assessing the influence of the microenvironment on cell function. For example, the role of hepatic stellate cells (HSC) on the long-term maintenance of hepatocytes has been studied using a liver-on-a-chip platform and demonstrated that the HSC plays a vital role in the formation of tight junction and enhancing liver functions. HSC influences hepatocyte function via cell-associated signalling and lipid transfer [40].

3.5 Heart-on-a-Chip for Cell Biology Studies

Heart-on-a-chip is another revolutionary application of microfluidics in which a minimal organoid heart is cultured in vitro with a closely controlled microenvironment that mimics human physiology. Conventional 2D cultures of cardiomyocytes limit the accuracy of predicted outcomes to different mechano-physio-chemical stimuli since the electrochemical behaviour of the heart is influenced by its 3D alignment and intercellular connections [41, 42]. Non-human animal model alternatives also bring interspecific variations in different molecular pathways [43], which in turn reduces the robustness of molecular screenings. Heart-on-a-chip models provide stable systems for all kinds of experiments with more accurate predictive power than conventional cell culture methods and in vivo non-human animal hearts.

The neurophysiology for a heart-on-a-chip device is carefully fabricated based on three major concepts: the three-dimensional alignment of tissue structure, the microcirculation, which facilitates nutrient exchange and transport and waste removal, and

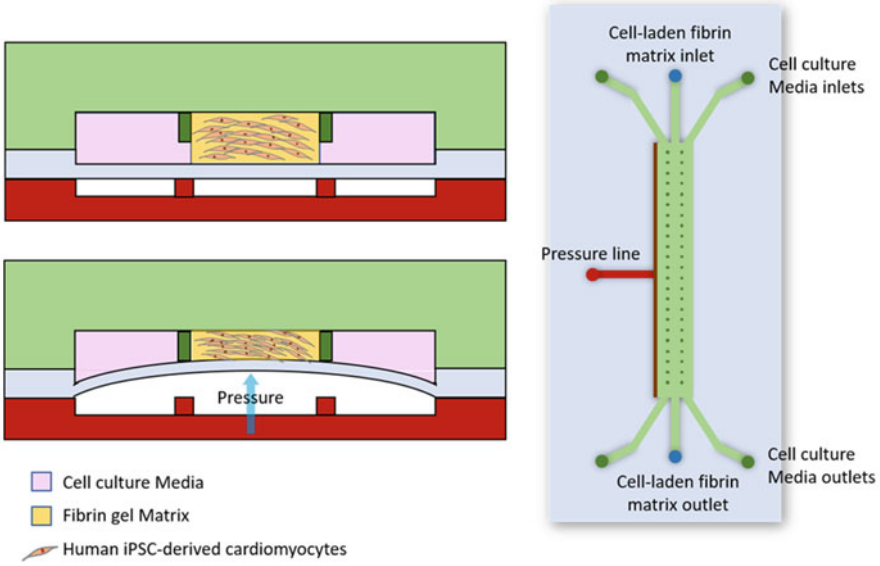

Fig. 3.1 Schematics of a heart-on-a-chip

shear flow protection of the cardiac tissue [44]. The microfluidic approach in organ-on-a-chip can precisely micromanage these essential concepts and mimic relevant physiology for the heart-on-a-chip platform. The key is to create a stable cardiomyocyte–vascular interface. The barrier within the interface is chosen carefully so that the perfusion through the 'vascular' microchannel allows a diffusion-based transport across rather than mass flow. The pressure of the flow is so kept that the diffusion rate mimics the in vivo condition. Closely manipulating the microenvironment enables cellular functions like excitation, cell–cell interactions (signalling) and cell–extracellular matrix interactions [45]. Different approaches are being employed to achieve this. For cardiomyocytes, differentiated and/or human-induced pluripotent stem cells (hiPSCs) can be used as cell sources. Several such heart-on-a-chip devices (Fig. 3.1) are developed according to specific needs like to study physiological alterations, disease models, drug and chemical screening, etc.

3.6 Intestine-on-a-Chip for Cell Biology Studies

The intestine is one of the critical organs that takes part in the absorption and digestion and harbours microbiome. It also communicates with the other organs through portal flow. The gut–brain axis formed by the enteric nervous system present in the intestine controls the ionic permeability, digestive function, mucosal secretion and transport. The microbes in the intestine help in maintaining intestinal homeostasis and also modulate the immune response. The coculture of microbes

with human intestine cells is necessary to study microbial interaction and intestinal homeostasis. However, conventional in vitro cell culture platforms are unable to maintain the long-term culture of intestinal epithelium and microbiomes. Owing to the vital functions in human physiology, many organ-on-a-chip models of the intestine have been developed recently. Most of the developed models of intestine-on-a-chip mimic the barrier function. The microfluidic models support the continuous perfusion of media and incorporation of physiological forces affecting the intestinal microenvironment, such as the peristalsis-like motion. The organ-on-a-chip models of the intestine have been shown to develop the essential intestine functions such as villi formation, and mucus production also enables long-time coculture with commensal microbes and pathogenic microbes. The studies using intestine-on-a-chip, which mimics the peristalsis-like movement, show that microbial growth greatly depends on the physiological forces. The introduction of mechanical forces can lead to spontaneous villus formation in the intestine-on-a-chip device characterised by proliferative cell restriction in basal crypts, reuptake of glucose, production of mucus and improved drug metabolising activity.

3.7 Lung-on-a-Chip for Cell Biology Studies

Respiratory-related diseases are one of the major causes of morbidity and mortality worldwide. Therefore, significant considerations have been given to developing lung disease models to study the basic cellular response and screen the therapeutics. Organ-on-a-chip models are one of the prominent systems that have been developed recently to recapitulate the organ-level function by considering the microenvironment niche and microphysiological forces present in the cellular vicinity. Successful models of lung-on-a-chip platforms of chronic obstructive pulmonary disease (COPD) [46], lung cancer [47, 48], asthma [49], acute respiratory tract infections, bronchitis, etc. have been developed so far.

Developing a successful lung-on-a-chip microfluidic system requires proper mimicking of complex lung physiology and suitable selection of cell type [50]. Primary cells or immortalised cells can be used to develop microfluidic cell culture systems. Primary cells such as the lung fibroblast [51], epithelial cells [52], alveolar epithelial cells [53], small airway epithelial cells (SAECs) [54] and lung smooth muscle cells [55] are directly isolated from the patient's tissue. The immortalised cell types such as A549 [56, 57], the adenocarcinoma epithelial cells, and NCI-H441 can be used to construct the lung-on-a-chip microfluidic devices [58]. Recently, induced pluripotent stem cells (iPSC) have been used to model lung diseases due to their ability to generate type II alveolar cells during directed differentiation. Since the lung airways and alveoli consist of multiple cell types, various cell combinations are necessary to obtain physiologically relevant results [59]. The selection of cells depends on the intended application. For example, to study idiopathic pulmonary fibrosis, Asami and co-workers used normal human lung fibroblast (NHLF) and small airway epithelial cells (SAECs) mixed with collagen type-1 to develop fibrotic microtissue [51]. Similarly, Zhang and co-workers used human pulmonary alveolar

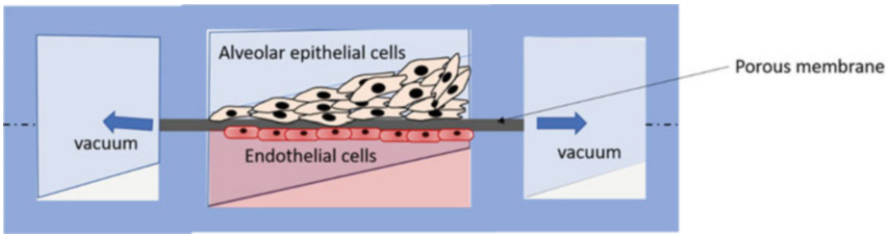

Fig. 3.2 Pictorial representation of lungs-on-a-chip

epithelial cells (HPAEpiCs) and human umbilical vein endothelial cells (HUVEC) to model nanoparticle exposure and study the pulmonary toxicity associated with the nanoparticle exposure by recreating the alveolar–capillary barrier [5].

Modelling the lung microenvironment requires proper mimicking of the dynamic behaviour of the lungs. Briefly, the lung cells are under continuous cyclic stretching due to respiration, and the cells also experience shear stress due to the blood perfusion. Also, the changes in oxygen partial pressure, and viscoelastic and stiffness variation of ECM across the lung, affect the phenotype and function of various cells present in the lungs. The air–liquid interface (ALI) in the alveolar epithelium also influences cellular activity. The microfluidic organ-on-a-chip approach of cell culture enables precise microenvironment control. The mimicking of cyclic stretching can be obtained by applying a vacuum in microfluidic devices (Fig. 3.2). Similarly, the air–liquid interface can be recreated by culturing the alveolar epithelial cells and endothelial cells on top and bottom of a thin stretchable porous membrane that separates two parallel microchannels. Such systems are valuable in understanding the effect of the physiological factors on the differentiation of epithelial progenitor cells, recruitment of macrophages during the infection, progression of pulmonary oedema, thrombosis, etc. [60].

Usually, the microfluidic devices are fabricated by soft-lithography using polydimethylsiloxane (PDMS) due to their ease of fabrication, optical visibility and biocompatibility. However, many studies pointed out the absorption of hydrophobic drugs to PDMS and altering of the drug efficacies. Hence, alternative materials such as hydrogel have been introduced to fabricate lung-on-a-chip devices.

Gelatin methacryloyl (GelMa) is one of the hydrogels that was successfully used to fabricate porous membranes [61]. The GelMa membranes are porous, and the stiffness lies in the range of native lungs. Importantly, it contains an arginine-glycine-aspartic acid sequence (RGD) and metalloproteinase-responsive peptide motifs, promoting cell adhesion and proliferation and migration. Also, GelMa can provide an alveolar sac-like structure, thereby properly mimicking the native lung alveolar structure. Alternatively, lamina propria can be developed by a three-chamber microfluidic system in which the epithelial cells and smooth muscle cells are separated by a middle chamber consisting of hydrogel containing Matrigel and collagen I, avoiding the use of a synthetic porous membrane [55]. The air–liquid interface is necessary for microfluidic models of the lung-on-a-chip; it will allow the

generation of differentiated epithelial cells and, hence, improve cellular functions. Proper mimicking of disease conditions in microfluidic devices will help understand pathophysiology associated with the disease progression. For example, the movement of liquid plugs through the airway associated with the disease condition causes compromised surfactant production [6, 62], such as cystic fibrosis and asthma. The oxygen gradient and tension occurring in the lung is also important while considering the lung's cellular activity. The oxygen levels exhibited in the lung directly impact the cellular proliferation, migration and differentiation of stem cells. The hypoxia condition developed in the cancer is a prime target in cancer therapy. Therefore, oxygen levels play a crucial role in the novel treatment strategies [57].

3.8 Kidney-on-a-Chip for Cell Biology Studies

The technologies developed in the field of molecular analytics, molecular biology, biodefence research and microelectronics are compiled together to develop microfluidic technology [63]. Organs-on-a-chip are microfluidic culture devices that are composed of a clear, flexible polymer that has hollow microfluidic channels that are lined with human organ cells and blood vessel cells. This technology helps to mimic the complex nature of native tissue both in vitro and ex vivo. Therefore, this technology can be utilised to study the kidneys and disorders related to kidneys. Nephrons are the structural and functional units of the kidney. The glomerulus, proximal convoluted tubule, distal convolute tube, collecting duct, etc. are different components of the nephron that can be mimicked using organ-on-a-chip technology to yield advancement in various fields, which includes testing ex vivo drug toxicity and renal replacement therapies [64].

The major advantage of microfluidic devices includes utilising a small quantity of reagent because of small dimensions, which in turn helps in cost-effective, highly efficient biochemical assay designing and diagnostics. Along with low reagent consumption, other advantages are high spatiotemporal resolution, high surface-to-volume ratio, portable system, etc. The high surface-to-volume ratio helps in the fast transport of mass and heat, which has wide applications in biotransformation. Microfluidic devices can be used to study single cells as it has a high spatiotemporal resolution. Single-cell types can be fixed in the device, and the physiology of the cells can be studied. A microenvironment can also be created using the device to mimic the complex nature of tissues as the concentration of different substrates used in developing such an environment can be adjusted by regulating laminar flow [65].

Polydimethylsiloxane (PDMS) is commonly used for fabricating microfluidic devices as it has the advantage of long shell life with greater permeability for gases and high chemical sensitivity and transparency. Hydrophobic compounds are absorbed by PDMS, which limits its application in microfluidic devices. Polycarbonate, polystyrene, etc. are different components in thermoplastic materials that have low absorption capacity for hydrophobic compounds and hence are often preferred in the formation of biochips. To both PDMS- and non-PDMS-based microfluidic devices, polymer-based hydrogels are added to mimic natural

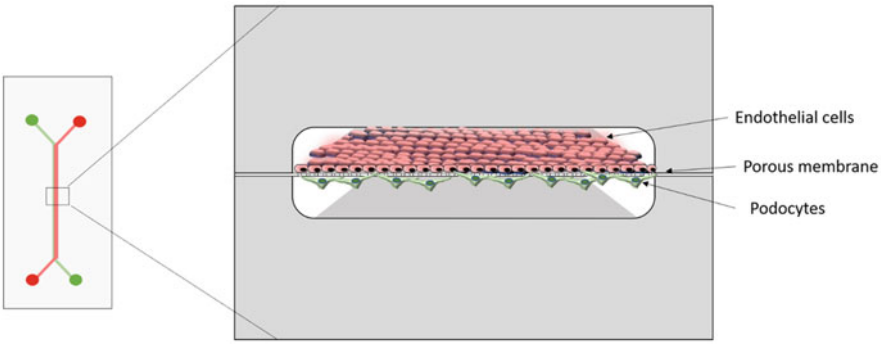

Fig. 3.3 Schematics of a glomerulus-on-a-chip model

physiological scaffolds. Within the bioengineered scaffolds, micro-sensors can be embedded to improve the assessment of physiological response to changes in the glomerular and tubular physiology environment.

Glomerulus-on-a-chip was used by Zhou et al. to develop the Hypertensive Nephropathy model. The glomerular endothelial cell that is conditionally immortalised and podocyte precursor cells of the mouse were used to line two microfluidic channels [66]. The channels are lined by layers of glomerular endothelial cells and podocytes that are in close approximation (Fig. 3.3). The cytoskeletal rearrangement in the adjacent cells was affected by the fluid flow in the channels that resulted in glomerular leakage and cellular damage. Similarly, Wang et al. reported the use of glomerulus-on-a-chip for studying early stage diabetic nephropathy [67].

Proximal tubular cells can also be used in the development of the microfluidic chip. Sciancalepore et al. cultured human progenitor cells of kidneys on polycarbonate membrane, which are porous in nature and are coated with fibronectin or laminin or Matrigel that separates microchannels [68]. In this study, it was found that the majority of the metabolically active cells were in association with fibronectin, and polarisation of cultured cells was induced by flow shear cells. Alternatively, a distal-tubule-on-a-chip developed by Régis Baudoin et al., using distal tubular cells of kidney (MDCK) cultured on fibronectin-coated microchannels, demonstrated the formation of a three-dimensional tissue-like structure [69].

3.9 Skin-on-a-Chip for Cell Biology Studies

Along with the progress in the development of various organ-on-a-chip platforms, in vitro models of skin were also developed. Studies including toxicity, efficacy, and drug targeting and delivery require physiologically relevant models of skin. Microfluidic models of skins enable the vascularised models of skin with dynamic perfusion [70]. Skin on the body acts as a physical barrier against various toxic and biological agents, including microorganisms, radiations, and nanoparticles. The in vitro models of skin that restore cellular functions are widely used for the

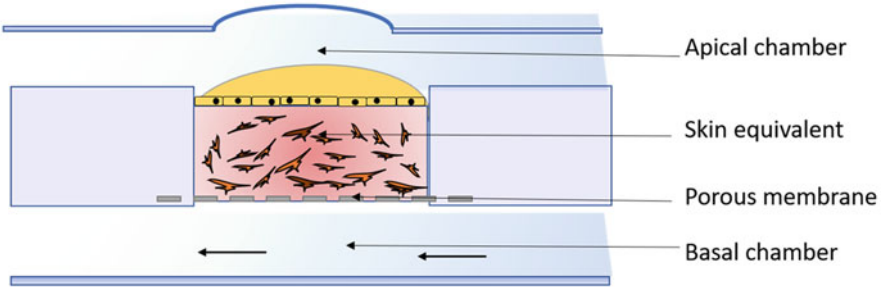

Fig. 3.4 Pictorial representation of a skin-on-a-chip device

high-throughput physiologically relevant toxicity screening of various substances and cosmetics. Initially, the skin-on-a-chip consisted of a cellular monolayer [71], but significant modifications were made later to generate more accurate models of native skin tissue. The skin can be generated on-chip by either direct introduction of tissue obtained from biopsy or by in situ generation. The compartmentalised skin-on-a-chip is widely used in research in which it allows a more realistic view of native tissue. Immune cell migration due to the presence of microbes was studied by the introduction of skin biopsy samples to one of the channels and another with blood samples of a microfluidic device in which each channel was separated by a red blood filter [72]. The transdermal transport occurs in a skin microtissue that can be studied with the aid of a microfluidic device in which the human skin equivalent is cultured on top of a porous membrane with media flow on the bottom channel [73]. Moreover, a skin biopsy can be used for the generation of multi-organ models; co-culturing of skin biopsies with other cells can increase the viability of the skin biopsies [74].

The in situ models of a skin-on-a-chip utilise the generation of skin tissue directly inside the chip. The mature skin can be developed in the microfluidic device (Fig. 3.4) by introducing fibroblast embedded in a collagen gel on top of a porous membrane followed by sequential seeding of keratinocytes above the gel [75]. Anti-ageing effects and the skin differentiation potential of various drugs have been successfully studied by such models [76]. Furthermore, the side effects of various drugs used for therapeutic purposes also can be analysed by skin-on-a-chip model [77]. The effect of mechanical stimuli on the skin was studied by Lim et al., using a microfluidic skin-on-a-chip platform, and resulted in the formation of wrinkles due to the stretching [78]. The dynamic conditions in the skin-on-a-chip are favourable to maintain viability for a longer period of time by reducing the growth rate significantly. Additionally, the barrier functions can also be modelled by using skin-on-a-chip platforms. Significant progress has been made in the development of skin-on-a-chip models, such as the fabrication of more complex tissue structures consisting of dermis, epidermis and blood vessels by a three-channel system with each channel separated by porous membranes. Inflammatory responses occurring due to the introduction of tumour necrosis factor-alpha (TNF-α) onto the dermal

compartment and further cytokine production have been successfully modelled using such systems.

3.10 Vascularised-Organ-on-a-Chip for Cell Biology Studies

Organ-specific heterogeneity of vasculature occurs at various levels: dimensions and architecture, flow rate and hence dynamic pressure, morphology and anatomy. These changes are largely linked to their specific functionalities. The development and modifications of the organ-specific vascular beds are mediated via chemical cues from the parenchymal cells of the organ; this factor also influences the morphology and anatomy of blood vessels. The difference in the microvasculatures associated with muscle and kidney is a striking example [79]. Another example of organ-specific heterogeneity is in the arrangement of the endothelial lining. According to classification done by Majno, there are three types of vessels: vessels with continuous endothelial lining (microvasculatures of skin, brain, skeletal muscles, lungs and myocardium); vessels with continuous endothelial lining having fenestrations (microvasculatures of endocrine glands and kidney); and vessels with discontinuous endothelial lining (microvasculature of liver and spleen) [80]. While recapitulating vascularised-organ-on-a-chip, to obtain biologically relevant data, it is important to reflect the organ-specific heterogeneities in the models. But the extent of mimicking heterogeneity highly depends on the objectives under investigation, as recapitulating all the in vivo conditions in a device are still a major challenge.

The sources of cells used for seeding the devices also vary greatly according to the objective of the study. Apart from the specific cells of the recapitulating organs, organ-specific endothelial cells (ECs) are obtained from different sources. Primary cells isolated from donors and human pluripotent stem cell (hPSC)-derived cells are the common sources of ECs. hPSCs are differentiated according to protocols prior to obtaining specific vascular ECs, which can then be seeded into organ-specific devices. This method has been followed by many research groups to build models of heart, blood-brain barrier [81], lungs [7], kidney [82] and liver [83]. Vasculature-on-a-chip is a device that supports vascular networks on microfluidic devices. A self-assembled vascular network is an approach that utilises the inherent capacity of the vascular cells to form a vasculature, with minimal control over the architecture of the networks formed and maximum biological relevance [84]. Developmental studies on vasculogenesis or tubulogenesis, vascular sprouting, etc. can be conducted with more fidelity using these models. The simplest approach is to culture endothelial cells in gel matrices like fibrin, collagen or Matrigel. Unlike using templates like needles as the mould for lumen, the ECs in gel use intracellular or extracellular mechanisms for tubulogenesis [85]. ECs can form intracellular lumen by aligning and fusing the vacuoles formed inside the adjacent cells or extracellular lumen by membrane invagination [86]. Other perivascular cells like pericytes can be added to the gel to support vasculogenesis and have been found to assemble a more biologically relevant basement membrane matrix.

3.11 Fabrication of Microfluidic Devices for Organ-on-a-Chip Experiment

The most common method of rapid prototyping of OOC is soft-lithography [87]. Soft-lithography starts with the spin-coating of the photoresist onto a clean silicon wafer, followed by soft-baking. Soft-lithography often requires a photomask. The microfluidic chip design in the photomask is transferred to the photoresist through ultraviolet (UV) exposure. UV light can pass through the transparent portions of the photomask, and the exposed area in the photoresist will polymerise. Subsequently, during the developing step, the unexposed or unpolymerised area leaves from the surface in the presence of etchant. The master mould can be further used for prototyping microfluidic devices. The degassed PDMS-curing agent solution (10:1) was cast onto the master mould, followed by thermal curing. The cured PDMS chip then peeled off from the photoresist. The fluidic connection requires inlet and outlet ports in the PDMS chip, which can be done through biopsy puncture. Cleaned devices can bond to various surfaces such as PDMS and glass. The bonding of PDMS to such surfaces requires surface treatment and the generally employed strategy is plasma activation of surfaces. The plasma treatment of PDMS/glass will generate hydrophilic surfaces, and it is suitable for irreversible binding. These devices require proper sterilisation before using for cell culture experiments. The PDMS devices are autoclavable and can perform cleaning by pumping with acetone/isopropanol/ethanol (70%), phosphate buffered saline (PBS) and by UV sterilisation.

3.12 Conclusion

Organ-on-a-chip enables the proper mimicking of the cellular microenvironment, which is necessary for predicting the cell function. Cells under various physiological conditions can behave differently. Therefore, the integration of microfluidic organ-on-a-chip devices into the preclinical studies can fast track the drug development process. Various physiological processes such as the fluid shear stress, peristalsis motion, cyclic stretching, formation of the air–liquid interface, the spatiotemporal gradient of the chemical, etc. can be incorporated into the microfluidic device. More importantly, individual cellular responses can also be studied using such a platform. However, to obtain physiologically relevant and meaningful data, proper selection of cell type, proper scaling of cells and incorporation of physiological forces in proper range are necessary.

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References

1. Bhatia SN, Ingber DE (2014) Microfluidic organs-on-chips. Nat Biotechnol 32:760–772. https://doi.org/10.1038/nbt.2989
2. Neuži P, Giselbrecht S, Länge K et al (2012) Revisiting lab-on-a-chip technology for drug discovery. Nat Rev Drug Discov 11:620–632. https://doi.org/10.1038/nrd3799
3. Esch MB, Smith AST, Prot J-M et al (2014) How multi-organ microdevices can help foster drug development. Adv Drug Deliv Rev 69:158–169
4. Zheng F, Fu F, Cheng Y et al (2016) Organ-on-a-Chip Systems: microengineering to biomimic living systems. Small 12:2253–2282
5. Zhang M, Xu C, Jiang L, Qin J (2018) A 3D human lung-on-a-chip model for nanotoxicity testing. Toxicol Res (Camb) 7:1048–1060
6. Tavana H, Zamankhan P, Christensen PJ et al (2011) Epithelium damage and protection during reopening of occluded airways in a physiologic microfluidic pulmonary airway model. Biomed Microdevices 13:731–742
7. Huh D (2015) A human breathing lung-on-a-chip. Ann Am Thorac Soc 12:S42–S44
8. Douville NJ, Zamankhan P, Tung Y-C et al (2011) Combination of fluid and solid mechanical stresses contribute to cell death and detachment in a microfluidic alveolar model. Lab Chip 11: 609–619
9. Cho S, Yoon J-Y (2017) Organ-on-a-chip for assessing environmental toxicants. Curr Opin Biotechnol 45:34–42. https://doi.org/10.1016/j.copbio.2016.11.019
10. Elias-Kirma S, Artzy-Schnirman A, Das P et al (2020) In situ-like aerosol inhalation exposure for cytotoxicity assessment using airway-on-chips platforms. Front Bioeng Biotechnol 8:91
11. Booth R, Kim H (2012) Characterisation of a microfluidic in vitro model of the blood-brain barrier (μBBB). Lab Chip 12:1784–1792. https://doi.org/10.1039/c2lc40094d
12. Grosberg A, Alford PW, McCain ML, Parker KK (2011) Ensembles of engineered cardiac tissues for physiological and pharmacological study: heart on-a-chip. Lab Chip 11:4165–4173
13. Agarwal A, Goss JA, Cho A et al (2013) Microfluidic heart on-a-chip for higher throughput pharmacological studies. Lab Chip 13:3599–3608
14. McCain ML, Sheehy SP, Grosberg A et al (2013) Recapitulating maladaptive, multiscale remodeling of failing myocardium on-a-chip. Proc Natl Acad Sci U S A 110:9770–9775
15. Ren L, Liu W, Wang Y et al (2013) Investigation of hypoxia-induced myocardial injury dynamics in a tissue interface mimicking microfluidic device. Anal Chem 85:235–244
16. Weinberg E, Kaazempur-Mofrad M, Borenstein J (2008) Concept and computational design for a bioartificial nephron-on-a-chip. Int J Artif Organs 31:508–514. https://doi.org/10.1177/039139880803100606
17. Jang K-J, Suh K-Y (2010) A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells. Lab Chip 10:36–42. https://doi.org/10.1039/B907515A
18. Rigat-Brugarolas LG, Elizalde-Torrent A, Bernabeu M et al (2014) A functional microengineered model of the human splenon-on-a-chip. Lab Chip 14:1715–1724. https://doi.org/10.1039/c3lc51449h
19. Kane BJ, Zinner MJ, Yarmush ML, Toner M (2006) Liver-specific functional studies in a microfluidic array of primary mammalian hepatocytes. Anal Chem 78:4291–4298
20. Du C, Narayanan K, Leong MF, Wan ACA (2014) Induced pluripotent stem cell-derived hepatocytes and endothelial cells in multi-component hydrogel fibers for liver tissue engineering. Biomaterials 35:6006–6014
21. Khetani SR, Bhatia SN (2008) Microscale culture of human liver cells for drug development. Nat Biotechnol 26:120–126
22. Wong SF, No da Y, Choi YY, Kim DS, Chung BG et al (2011) Concave microwell based size-controllable hepatosphere as a three-dimensional liver tissue model. Biomaterials 32:8087–8096

23. Ma C, Zhao L, Zhou E-M et al (2016) On-chip construction of liver lobule-like microtissue and its application for adverse drug reaction assay. Anal Chem 88:1719–1727. https://doi.org/10.1021/acs.analchem.5b03869
24. Park J, Lee BK, Jeong GS et al (2015) Three-dimensional brain-on-a-chip with an interstitial level of flow and its application as an in vitro model of Alzheimer's disease. Lab Chip 15:141–150. https://doi.org/10.1039/c4lc00962b
25. Jeong GS, Chang JY, Park JS et al (2015) Networked neural spheroid by neuro-bundle mimicking nervous system created by topology effect. Mol Brain 8:1–12
26. Swain JE, Smith GD (2011) Advances in embryo culture platforms: novel approaches to improve preimplantation embryo development through modifications of the microenvironment. Hum Reprod Update 17:541–557
27. Bhise NS, Manoharan V, Massa S et al (2016) A liver-on-a-chip platform with bioprinted hepatic spheroids. Biofabrication 8:14101
28. Murphy SV, Atala A (2014) 3D bioprinting of tissues and organs. Nat Biotechnol 32:773–785
29. Bertassoni LE, Cardoso JC, Manoharan V et al (2014) Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels. Biofabrication 6:24105. https://doi.org/10.1088/1758-5082/6/2/024105
30. Haüssinger D (1988) Hepatic sublobular synthesis of urea: control by oxygen or by pH gradient? Hepatology 8:184–186. https://doi.org/10.1002/hep.1840080134
31. Li CY, Stevens KR, Schwartz RE et al (2014) Micropatterned cell-cell interactions enable functional encapsulation of primary hepatocytes in hydrogel microtissues. Tissue Eng Part A 20:2200–2212. https://doi.org/10.1089/ten.tea.2013.0667
32. Esch MB, Prot J-M, Wang YI et al (2015) Multi-cellular 3D human primary liver cell culture elevates metabolic activity under fluidic flow. Lab Chip 15:2269–2277. https://doi.org/10.1039/c5lc00237k
33. Tostões RM, Leite SB, Serra M et al (2012) Human liver cell spheroids in extended perfusion bioreactor culture for repeated-dose drug testing. Hepatology 55:1227–1236. https://doi.org/10.1002/hep.24760
34. Ho WJ, Pham EA, Kim JW et al (2010) Incorporation of multicellular spheroids into 3-D polymeric scaffolds provides an improved tumor model for screening anticancer drugs. Cancer Sci 101:2637–2643. https://doi.org/10.1111/j.1349-7006.2010.01723.x
35. Ho CT, Lin RZ, Chen RJ et al (2013) Liver-cell patterning Lab Chip: mimicking the morphology of liver lobule tissue. Lab Chip 13:3578–3587. https://doi.org/10.1039/c3lc50402f
36. Du Y, Li N, Yang H et al (2017) Mimicking liver sinusoidal structures and functions using a 3D-configured microfluidic chip. Lab Chip 17:782–794. https://doi.org/10.1039/C6LC01374K
37. Du Y, Khandekar G, Llewellyn J et al (2020) A bile duct-on-a-chip with organ-level functions. Hepatology 71:1350–1363. https://doi.org/10.1002/hep.30918
38. Yu F, Goh YT, Li H et al (2020) A vascular-liver chip for sensitive detection of nutraceutical metabolites from human pluripotent stem cell derivatives. Biomicrofluidics 14:034108
39. Ma L-D, Wang Y-T, Wang J-R et al (2018) Design and fabrication of a liver-on-a-chip platform for convenient, highly efficient, and safe in situ perfusion culture of 3D hepatic spheroids. Lab Chip 18:2547–2562. https://doi.org/10.1039/C8LC00333E
40. Krause P, Saghatolislam F, Koenig S et al (2009) Maintaining hepatocyte differentiation in vitro through co-culture with hepatic stellate cells. In Vitro Cell Dev Biol Anim 45:205–212. https://doi.org/10.1007/s11626-008-9166-1
41. Costa KD, Takayama Y, McCulloch AD, Covell JW (1999) Laminar fiber architecture and three-dimensional systolic mechanics in canine ventricular myocardium. Am J Physiol Circ Physiol 276:H595–H607
42. LeGrice IJ, Smaill BH, Chai LZ et al (1995) Laminar structure of the heart: ventricular myocyte arrangement and connective tissue architecture in the dog. Am J Physiol Circ Physiol 269:H571–H582
43. Scott CW, Peters MF, Dragan YP (2013) Human induced pluripotent stem cells and their use in drug discovery for toxicity testing. Toxicol Lett 219:49–58

44. Mathur A, Loskill P, Shao K et al (2015) Human iPSC-based cardiac microphysiological system for drug screening applications. Sci Rep 5:1–7. https://doi.org/10.1038/srep08883
45. Ribas J, Sadeghi H, Manbachi A et al (2016) Cardiovascular organ-on-a-chip platforms for drug discovery and development. Appl In Vitro Toxicol 2:82–96
46. Benam KH, Villenave R, Lucchesi C et al (2014) Development of a human COPD model-on-a-chip to mimic disease exacerbation (a small airway-on-a-chip model). Eur Respir J 44:3340
47. Liao H-J, Chieh J-A, Chen Y-C et al (2021) Lung cancer on chip for testing immunotherapy. In: 2021 21st international conference on solid-state sensors, actuators and microsystems (transducers). IEEE, New York, pp 1032–1035
48. Park S, Kim TH, Kim SH et al (2021) Three-dimensional vascularised lung cancer-on-a-chip with lung extracellular matrix hydrogels for in vitro screening. Cancers (Basel) 13:3930
49. Nesmith AP, Agarwal A, McCain ML, Parker KK (2014) Human airway musculature on-a-chip: an in vitro model of allergic asthmatic bronchoconstriction and bronchodilation. Lab Chip 14:3925–3936
50. Bennet TJ, Randhawa A, Hua J, Cheung KC (2021) Airway-on-a-chip: designs and applications for lung repair and disease. Cells 10:1602
51. Asmani M, Velumani S, Li Y et al (2018) Fibrotic microtissue array to predict anti-fibrosis drug efficacy. Nat Commun 9:1–12
52. Rayner RE, Makena P, Prasad GL, Cormet-Boyaka E (2019) Optimisation of normal human bronchial epithelial (NHBE) cell 3D cultures for in vitro lung model studies. Sci Rep 9:1–10
53. Bluhmki T, Bitzer S, Gindele JA et al (2020) Development of a miniaturised 96-Transwell air–liquid interface human small airway epithelial model. Sci Rep 10:1–14
54. Benam KH, Mazur M, Choe Y et al (2017) Human lung small airway-on-a-chip protocol. In: 3D cell culture. Springer, New York, pp 345–365
55. Humayun M, Chow C-W, Young EWK (2018) Microfluidic lung airway-on-a-chip with arrayable suspended gels for studying epithelial and smooth muscle cell interactions. Lab Chip 18:1298–1309
56. Sallin P, Barbe L, Gazdhar A et al (2011) Lung on chip: in vitro HGF effects on injured alveolar A549 epithelial cells in microfluidic system. Eur Respir J 38:4584
57. Yang X, Li K, Zhang X et al (2018) Nanofiber membrane supported lung-on-a-chip microdevice for anti-cancer drug testing. Lab Chip 18:486–495
58. Cidem A, Bradbury P, Traini D, Ong HX (2020) Modifying and integrating in vitro and ex vivo respiratory models for inhalation drug screening. Front Bioeng Biotechnol 8:1256
59. Jacob A, Morley M, Hawkins F et al (2017) Differentiation of human pluripotent stem cells into functional lung alveolar epithelial cells. Cell Stem Cell 21:472–488
60. Huh D, Leslie DC, Matthews BD et al (2012) A human disease model of drug toxicity–induced pulmonary edema in a lung-on-a-chip microdevice. Sci Transl Med 4:159ra147
61. Huang D, Liu T, Liao J et al (2021) Reversed-engineered human alveolar lung-on-a-chip model. Proc Natl Acad Sci U S A 118(19):e201614611
62. Huh D, Fujioka H, Tung Y-C et al (2007) Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems. Proc Natl Acad Sci U S A 104:18886–18891
63. Wu Q, Liu J, Wang X et al (2020) Organ-on-a-chip: recent breakthroughs and future prospects. Biomed Eng Online 19:1–19
64. Ashammakhi N, Wesseling-Perry K, Hasan A et al (2018) Kidney-on-a-chip: untapped opportunities. Kidney Int 94:1073–1086
65. Ortseifen V, Viefhues M, Wobbe L, Grünberger A (2020) Microfluidics for biotechnology: bridging gaps to foster microfluidic applications. Front Bioeng Biotechnol 8:1324
66. Zhou M, Zhang X, Wen X et al (2016) Development of a functional glomerulus at the organ level on-a-chip to mimic hypertensive nephropathy. Sci Rep 6:1–13
67. Wang L, Tao T, Su W et al (2017) A disease model of diabetic nephropathy in a glomerulus-on-a-chip microdevice. Lab Chip 17:1749–1760

68. Sciancalepore AG, Sallustio F, Girardo S et al (2014) A bioartificial renal tubule device embedding human renal stem/progenitor cells. PLoS One 9:e87496
69. Baudoin R, Griscom L, Monge M et al (2007) Development of a renal microchip for in vitro distal tubule models. Biotechnol Prog 23:1245–1253
70. Risueño I, Valencia L, Jorcano JL, Velasco D (2021) Skin-on-a-chip models: general overview and future perspectives. APL Bioeng 5:30901
71. Suhail S, Sardashti N, Jaiswal D et al (2019) Engineered skin tissue equivalents for product evaluation and therapeutic applications. Biotechnol J 14:1900022
72. Kim JJ, Ellett F, Thomas CN et al (2019) A microscale, full-thickness, human skin on-a-chip assay simulating neutrophil responses to skin infection and antibiotic treatments. Lab Chip 19: 3094–3103
73. Abaci HE, Gledhill K, Guo Z et al (2015) Pumpless microfluidic platform for drug testing on human skin equivalents. Lab Chip 15:882–888. https://doi.org/10.1039/c4lc00999a
74. Ataç B, Wagner I, Horland R et al (2013) Skin and hair on-a-chip: in vitro skin models versus ex vivo tissue maintenance with dynamic perfusion. Lab Chip 13:3555–3561
75. Song HJ, Lim HY, Chun W et al (2017) Fabrication of a pumpless, microfluidic skin chip from different collagen sources. J Ind Eng Chem 56:375–381
76. Kim J, Kim K, Sung GY (2020) Coenzyme Q10 efficacy test for human skin equivalents using a pumpless skin-on-a-chip system. Int J Mol Sci 21:8475
77. Jeon HM, Kim K, Choi KC, Sung GY (2020) Side-effect test of sorafenib using 3-D skin equivalent based on microfluidic skin-on-a-chip. J Ind Eng Chem 82:71–80
78. Lim HY, Kim J, Song HJ et al (2018) Development of wrinkled skin-on-a-chip (WSOC) by cyclic uniaxial stretching. J Ind Eng Chem 68:238–245
79. Bassenge E (1996) Endothelial function in different organs. Prog Cardiovasc Dis 39:209–228. https://doi.org/10.1016/S0033-0620(96)80002-8
80. Risau W (1998) Development and differentiation of endothelium. Kidney Int Suppl 54:3–6. https://doi.org/10.1046/j.1523-1755.1998.06701.x
81. Lee CS, Leong KW (2020) Advances in microphysiological blood-brain barrier (BBB) models towards drug delivery. Curr Opin Biotechnol 66:78–87. https://doi.org/10.1016/j.copbio.2020.06.009
82. Wilmer MJ, Ng CP, Lanz HL et al (2016) Kidney-on-a-chip technology for drug-induced nephrotoxicity screening. Trends Biotechnol 34:156–170. https://doi.org/10.1016/j.tibtech.2015.11.001
83. March S, Hui EE, Underhill GH et al (2009) Microenvironmental regulation of the sinusoidal endothelial cell phenotype in vitro. Hepatology 50:920–928. https://doi.org/10.1002/hep.23085
84. Pollet AMAO, den Toonder JMJ (2020) Recapitulating the vasculature using Organ-on-Chip technology. Bioengineering 7:1–18. https://doi.org/10.3390/bioengineering7010017
85. Davis GE, Stratman AN, Sacharidou A, Koh W (2011) Molecular basis for endothelial lumen formation and tubulogenesis during vasculogenesis and angiogenic sprouting, 1st edn. Elsevier Inc., Amsterdam
86. Kamei M, Brian Saunders W, Bayless KJ et al (2006) Endothelial tubes assemble from intracellular vacuoles in vivo. Nature 442:453–456. https://doi.org/10.1038/nature04923
87. Puryear JR III, Yoon J-K, Kim Y (2020) Advanced fabrication techniques of microengineered physiological systems. Micromachines 11:730

Microfluidics in Chemical Biology

4

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Abstract

Chemical biology is the field where two scientific disciplines, that is, chemistry and biology, join. Techniques derived from chemistry are used to study or manipulate biological or natural products. Chemical biology helps develop bioassays for the quantification of various compounds. The wisdom from chemistry is used to develop techniques to purify proteins and nucleic acids or use small molecules for multiple applications, such as sensing, drug discovery, tissue engineering, disease modelling and molecular genetics. The 1970s witnessed the development of a novel field of engineering that manipulates micro- or nanoquantities of fluids using channels and pumps for specific applications called microfluidics. The development of various microfabrication techniques and novel materials in the past few decades has led to efficient microfluidic platforms. The convergence of microfluidics, chemistry and biology gives us a platform where minute quantities of samples are orchestrated to ensemble an overall result that helps researchers in various scientific fields. This chapter briefly discusses the application of microfluidics in chemical biology in bioassays, separation and purification of proteins and nucleotides, molecular self-assembly, tissue engineering and nucleotide sequencing. These aspects include exposure to electrophoresis and chromatography in microfluidic devices, gradients in microfluidic devices, surface modification strategies, and polymerase chain reaction (PCR).

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4.1 Introduction

Chemical biology is the convergence of chemistry and biology. It involves the study of reactions concerning biological processes. Chemical biology deals with reactions involving biomolecules such as lipids, carbohydrates and proteins and small molecules such as metals, probes and drugs which shine a light on the basic working of a living body. Chemical biology is also of paramount importance to researchers since it forms the basis for various laboratory techniques. It is impossible to obtain outcomes for procedures such as protein extraction, purification and crystallization; manipulation of different cell lines; synthesis of non-natural probes; and molecular self-assembly without chemical biology. Since chemical biology is unavoidable in almost any life science laboratory, improvements in platforms to carry out this field will aid researchers in carrying out works in a much more efficient manner. Microfluidic platforms are a robust means to reinforce the research carried out in the field by reducing the samples required and improving overall efficiency.

Generally, microfluidics is the manipulation of fluid flow in quantities from microlitres to as small as femtolitres with the help of micro-channels, valves and pumps to yield relevant results. The channels are imprinted onto a polymer platform using techniques such as soft lithography, chemical etching, hot embossing, micro-moulding, 3D printing, reactive ion etching and micromachining [1]. The most commonly used polymers are polydimethylsiloxane (PDMS), poly lactic-co-glycolic acid (PLGA), polylactic acid (PLA) and poly(methyl methacrylate) (PMMA) [2]. Glass and silicon are other non-polymer materials employed for fabrication. The materials are selected based on the properties such as tensile strength, elasticity index, plasticity, hardness, electrical and mechanical conductivity, inertness, gas permeability, liquid permeability and transparency to visible and UV light. The material used depends on the need in chemical biology. In chemical biology, microfluidics reduces the sample size significantly, requiring very little reagent while enabling easy reaction monitoring. The reactions performed in these devices are extrapolated easily for a full-scale model. The device also permits us to mimic biochemical reactions with better efficiency as the intrinsic dimensions of the device channel are comparable to that of a eukaryotic cell. The device also provides a better environment since it is easy to maintain pressure and other mechanical motion parameters.

4.2 Microfluidics in Analytical Chemistry

Analytical chemistry includes the convergence of various techniques that examine various aspects and purposes of a chemical reaction; this can be in chemistry, life science, or biochemistry. Analytical chemistry is broadly classified into separations and reactions depending on the course of action [3]. Applications of microfluidics in the field of analytical chemistry have innumerable advantages. A somewhat laborious and time-consuming protocol is performed with significant ease using very little time. This advantage is even more notable in life sciences as the reactants are usually less in quantity and costly. Hence, the above advantage increases the efficiency of the work. Another advantage of using a microfluidic platform is the possible precision control of behaviour in the system, thus enabling fastidious reactions using microfluidic platforms. Microfluidic devices can work without much human intervention, allowing the safe use of toxic reagents without human harm.

4.2.1 Microfluidic Devices in Chemical Reactions

The previous decade saw an enormous boom in the development of microfluidic devices for various purposes in research. Even though end consumers rarely use microfluidic devices commercially, several companies manufacture devices directly used in the laboratory [3–5]. Microfluidic devices are used for various applications, such as DNA analysis, chemical synthesis, enzymatic reactions and many other immunoassays.

The field of life science brings its challenges, one of which is the availability of reagents and samples. Since significantly less quantity of sample is available for studies, a platform that uses samples and reagents in the femtomolar range brings down the overall cost of the process and increases the efficiency. A microfluidic device employing silica beads and photodiodes designed to detect genetic material in the femtomolar range using sample volumes as low as 5 μL is developed [6]. Another PDMS-glass device using minimal sample volumes, employing micropillars and aptamers, was used to capture cancer cells [7]. Yang et al. demonstrated a device for isolating single cancer cells from whole blood [8]. The detection for both the above cases was done using fluorescence.

In contrast to fluorescence- and chemiluminescence-based detection, colorimetric detection is significantly simple. The costly probes are not required, and the detection devices are easier to design. A disadvantage of using colorimetry is that it cannot provide high-resolution images. However, it is practically nullified as the colorimetry devices employ a high-resolution camera-integrated smartphone allowing a point-of-care approach [6, 9]. This point-of-care approach has led to various lab-on-a-chip devices to analyse several components in the human body. Paper-based microfluidic devices are employed widely for the detection of anything from ions [10, 11] to components such as glucose and proteins [12], lactate [13], uric acid [14], total amino acid content [15], urea [16] and other biomarkers [17, 18]. The paper-based microfluidic device can detect the components mentioned above from

body fluids such as whole blood, plasma, urine, sweat and saliva [17, 19–21]. Interestingly, instead of using a smartphone, techniques are developed for reading data using a handheld device by transmitting light through paper-based microfluidic devices [22]. Researchers have employed paper-based microfluidic devices to create a system to detect multiple components from a single sample [17, 23].

4.3 Separation and Purification of Biomolecules

Chromatography and electrophoresis are analytical and separation procedures. They are widely utilized for macromolecules' macroscale separation and evaluation, namely proteins and nucleic acids. Chromatography can isolate a wide range of particles by the appropriate stationary phase and the presence of distinct ligands. In contrast, electrophoresis offers the detachment of charged species as per the ratio of molecule charge/size upon the utilization of an electric field. The two strategies provide the likelihood to recognize the structure and properties of a biomolecule. However, they have likewise tedious protocols and high costs, additionally at times require laboratory infrastructure and talented staff. On miniaturizing the two methods, such disadvantages are overcome [24].

4.3.1 Electrophoresis

Approaches of microfluidic chip electrophoresis for the separation of biomolecules.

4.3.1.1 Microchip Capillary Electrophoresis

Capillary electrophoresis (CE) has turned into a powerful and functional analysis method with benefits of electrolyte utilization of low sample volume, quick analysis time, simple coupling with other sensitive detection techniques, and good separation efficiency [25]. Distinctive separation methods for isolation and identification of different biochemicals include capillary zone electrophoresis (CZE), capillary isoelectric focusing (CIEF), micellar electrokinetic chromatography (MEKC), capillary isotachophoresis (CITP) and non-gel sieving capillary electrophoresis (NGS-CE) [26]. Capillary zone electrophoresis is a dominantly used separation method on microprocessors among the capillary electrophoresis for charged species [27].

MEKC separates neutral and charged species based on partitioning between micelle and background electrolyte and electrophoretic mobility. High proficiency serpentine PDMS microfluidic chips using fluorescent proteins are helpful in MEKC separation. In MEKC, functionalized ionic liquid determines the protein separation, which could be utilized as a surface modifier and supporting electrolyte to prevail the adsorption of analytes [28].

CIEF is a notable and predominant separation strategy for protein separation. It is accomplished with monolithic immobilized pH gradient materials in microchannels that are introduced to isolate ribonuclease B, α-casein and myoglobin [29]. In addition, the isolation of nucleic acids utilizing a low sample of about 25 μL of

CITP is also an innovative microchip [30]. Another microdevice utilizing NGS-CE, loaded with short linear polyacrylamide solution, identifies the DNA fragments isolated from the gastric cancer tissue using restriction endonuclease fingerprinting. It finishes DNA detection with the help of laser-induced fluorescence (LIF) in a short period [31]. One-dimensional division mode lacks peak capacity, making it harder to examine the complex biological samples with a satisfactory resolution. Significant commitments to take advantage of a two-dimensional mode of separation on a microfluidic device for peptide blends or complex proteins analysis improve coupling MEKC accompanied by high field strength CE, which enhances the quick separation of peptides processed from a model tryptase [32].

4.3.1.2 Microchip Gel Electrophoresis

Microfluidic gel electrophoresis facilitates all sample preparing steps automatically in a miniaturized channel with reagents of diminished volumes. Furthermore, it allowed the joule heating to dissipate rapidly and advanced the improvement of gel electrophoresis [33]. In isolation of nucleic acids, agarose gel acts as a better sieving matrix for electrophoresis. Additionally, an ultrahigh-throughput way to investigate the genomic damage of cells with excellent reproducibility is developed using agarose-based microfluidic devices [34]. The isolation of DNA on polyester-toner (PeT) based electrophoresis microchip is successfully studied. On this PeT chip, channels loaded with hydroxypropyl cellulose or hydroxyethyl cellulose help isolate DNA fragments with good separation efficiency. Further studies concluded that the microdevice filled with the solutions of short straight polymer dissipated the joule heating rapidly, which prolonged the life cycle of the microfluidic device [35].

4.3.2 Chromatography

Chromatographic techniques proficiently separate biomolecules in an unadulterated manner after the pre-treatment of the sample. However, it requires substantial sample volumes and is relatively time-devouring. Microfluidic chips attained consideration from the researcher community as an option to conquer the inadequacies of conventional gadgets. A microfluidic chip can be envisioned as a miniaturized device of multiplexed channels in an organized network [36]. Specifically, isolation of biomolecules is carried out by different chromatography procedures such as affinity chromatography (AC), size exclusion chromatography (SEC), hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IEX) [26]. The incorporation of stationary phases within the microchannels is crucial in chromatography for the isolation of molecules. Commonly used stationary phase materials are monoliths, resins and membranes. Nanowires (NWs) are also inserted into the microfluidic chips for biomolecule isolation along with these stationary phases. Moreover, pillar structured and surface functionalized microchannels like open channel frameworks have been utilized as stationary phases [36].

4.3.2.1 Separation of Nucleic Acids

Extraction of nucleic acids with the help of chaotropic agents, namely guanidinium thiocyanate, from various complex combinations are also extended to use in microfluidic chips. A common practice is the specific isolation of nucleic acids in an aqueous medium utilizing affinity ligands like human immunoglobulin E. The outcomes revealed that the essential requirement of affinity ligands is economically suitable and can adsorb nucleic acids selectively from complex mixtures [36].

4.3.2.1.1 Open-Channel Microfluidic Chips

Microfabrication is one of the innovations for fabricating microchannels and microstructures using various materials. Glass and silicon microfluidic chips are fabricated in a cleanroom atmosphere utilizing thin-film metallization, photolithography and chemical etching. Limitations of silicon and glass and expanding requests for the minimal expense of plastic and polymer materials have prompted the next-generation fabrication techniques. Both pillar and open structured channels are used for the stationary phase for biomolecule extraction [36].

Modification of functional groups is done on microchannel surface with cyclic olefin polymer (COP), namely diethylene glycol dimethyl ether (DEGDME), polyethylene glycol (PEG) with a neutral charge, bovine serum albumin (BSA) with a negative charge, and (3-aminopropyl)triethoxysilane (APTES) with a positive charge on the open-channel microchips. Adsorption of ssDNA atom is further examined on both altered and unaltered microfluidic chips. The negatively charged DNA follows affinity as APTES > PEG > BSA. If an occurrence of PEG appended COP should arise, electrostatic associations play a significant part in the affinity towards DNA. The substrate covered with BSA shows less affinity towards DNA. An increment of the microchannel length improves the DNA adsorption limit [37].

A dispensable PDMS microchip with engraved glass isolates DNA of varying lengths in a reduced electric field. This microchip effectively separated DNA fragments from mixtures and offered benefits, including re-using and separation efficiency with minimal loss [38]. Cyclic olefin copolymer (COC) and PMMA microchips are manufactured by exploring the injection moulding method. The principle of isotachophoresis (ITP) is good to isolate nucleic acids from an organic sample of salmon sperm and whole human blood. Even though the presentation of the two materials is comparative, COC needs extra pressure to move it in through the microchannel unlike other materials which spontaneously move through the channels [39].

4.3.2.1.2 Pillar Structured Microfluidic Chips

In this, augmentation of the microchannel surface region consolidates fabricating shapes in the form of pillars with the help of microfabrication techniques [36].

A silicon dioxide-coated silicon-PDMS microchip is employed to separate RNA. The dispensable PDMS cartridge contains repositories that can hold reagents, a microchannel that allows reagents' movement, and a pin valve that controls the fluid flow. The microchannel interface of the reservoir determines the bonding strength, which is explored by subjecting the microfluidic chip to an average

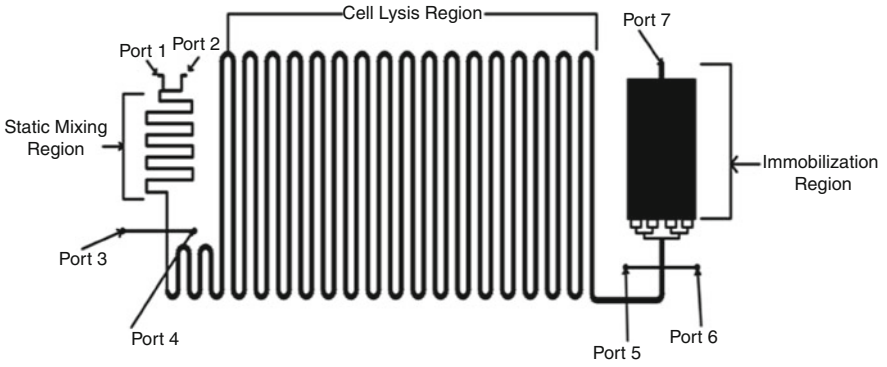

Fig. 4.1 An integrated microfluidic device for on-chip cell lysis and affinity extraction of RNA

pressure. The shortfall in liquid leakage demonstrated the bonding strength of the substrates. This chip separated dengue infection serotype 2 RNA (DEN2 RNA) from a pre-lysed infection culture [40]. A coordinated Pyrex 7740 glass microchip isolates RNA, as shown in Fig. 4.1. Crown designs are immobilized with specific oligonucleotides in the extraction chamber. Rhodamine marked IL-13 rRNAs show affinity to the oligonucleotide, affirmed by the fluorescence magnifying lens pictures. The weak bonding of RNA with oligonucleotide resulted in the effective elution of RNA on the application of heat [41].

'Bowed edges' inside miniature channels of PDMS microfluidic chip accomplished the fast isolation of DNA. The model combinations of minicircle DNA and linear DNA fragments demonstrated the separation efficiency along with parental DNA. Low-weighted DNA fragments passed the ridge without any hindrance, while those with more weight diverted to reach the ridge towards the contrary channel divider when the continuous injection was applied [42].

4.3.2.1.3 Resin Incorporated Microfluidic Chips

The resins for chromatography include polymers, inorganic materials and natural polysaccharides. Furthermore, the materials are modified with various chemistries to immobilize the ligands. Resins used for chromatography have a large surface area and adsorption capacity. However, their application at the analytical scale is limited due to mechanical weakness, time-consuming packing procedure, poor mass transfer and significant pressure build-up [36]. The most important strategy is to include chromatography resins such as silica resins and others into the microchannels of microfluidic devices. To firmly retain the resins, some researchers utilized 'weir structures' with different heights. Others used a polyethylene membrane as a 'Frit substance' that contained the silica resins inside the microchannel confines. An intriguing tactic during the extraction tests was the utilization of the sol-gel as an 'interparticle glue' to keep the resins intact, maintain consistent packing and minimize packed bed compression [36].

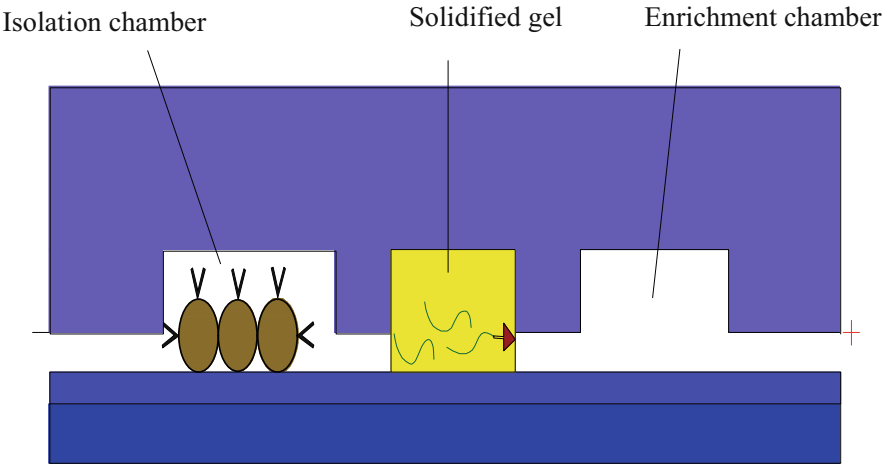

Fig. 4.2 IgE functionalized microbeads withheld within microchannels using weir structure

The affinity chromatography concept was applied in a glass-PDMS hybrid microchip for the isolation of ssDNA selectively. Separation, transfer and enrichment chambers are the conjoined chambers of microchannel on the microfluidic chip, as depicted in Fig. 4.2. The first microchannel chamber is filled with human IgE immobilized microbead to select ssDNA from a randomized ssDNA mixture. The thermally eluted ssDNA strands are transported to the third microchannel chamber through electrophoresis in the second chamber containing solidified agarose gel. Furthermore, changing the old IgE immobilized beads with new ones enhanced the efficiency of the multiple uses of the device. The benefit of this microfluidic gadget over existing gadgets is the likelihood to consolidate electrophoresis and solid-phase extraction on a solitary chip [43].

Another glass microchip with MagneSil™ particles isolates DNA from enormous sample volumes obtained from clinical labs. Human and mitochondrial DNA was extracted successfully from dilute whole blood and deteriorated bloodstains, demonstrating the microchip's extraction effectiveness. The overall biological sample volume is considerably low, while the concentration of DNA is high. This framework impedes the potential contamination with the co-eluted PCR inhibitors [44].

4.3.2.1.4 Monolith Incorporated Microfluidic Chips

According to the definition, a monolith is a single piece of continuous stationary phase with a strongly linked porosity network. Flow is convective due to strong pore interconnectivity, resulting in a faster mass transfer of molecules and quick separation. Monoliths are inorganic or organic based on the characterization. Monolith integration in microfluidic devices was sparked by technological advantages such as ease of introduction into microchannels as a homogeneous solution, in situ polymerization, and the absence of Frits [36]. Another extra element is the likelihood to set

them up at a particular site on the microchannel by photograph-initiated polymerization helped with customized photomasks. The ideal functionality of monoliths is accomplished by utilizing two methodologies. The primary method is a multi-step measure that modifies the chemical moieties on a monolith that has been pre-synthesized. The subsequent methodology is a solitary advance interaction that includes the monomer addition functionalized with sulfonate, methyl, or carboxylate to the solution of co-monomer and crosslinker atoms before in situ polymerization [36].

A silica monolith microchip separated DNA from a low cell number sample. Here, DNA binds in an irreversible method to silica monolith when present at low concentrations. However, the increment of concentration of DNA load reduces this pattern due to the filling of reversible destinations preferentially [45]. Another coordinated microchip isolated DNA from buccal cells under chaotropic conditions uses silica monolith as the stationary phase. This chip contains low-melting temperature (LMT) agarose gel to hold the reagents for DNA extraction and polymerase chain reaction (PCR) intensification. Liquids head to the microchip utilizing the electro-osmotic pumping (EOP) rule. The main benefit of the framework is storing reagents without critical loss and appropriate processing of clinical samples [46].

4.3.2.1.5 Nanowires Incorporated Microfluidic Chips

Nanowire (NW) structures make spatial and controlled nanostructures. SnO_2 nanowires joined inside microfluidic gadgets have been utilized to extract biomolecules based on size exclusion chromatography. Self-assembly technology assisted in the production of nanowire spot array structures in the fused silica microchannels. This device is more suitable for separating long DNA molecules when contrasted with a nanowall array microchip. Three-dimensional inflexible nanowire, when integrated inside the microchannel utilizing vapour-liquid-solid (VLS) technique, showed that nanowire structures with high density have better separation efficiencies than existing nanopillar structures in proficiently isolates various sizes of DNA [36]. Protein and RNA are separated from the three-dimensional rigid nanowire microchip upon increasing the nanowire's growth cycle by reducing the pore size distribution of nanowires. This device's advantage is the likelihood of controlling pore size dispersion between nanowires by characterizing the quantity of nanowire development cycles. These chips have proficient biomolecule separation because of the presence of the SiO_2 layer and the applied electric field getting concentrated inside the pores of firm nanowire structures [47].

4.3.2.2 Separation of Proteins

4.3.2.2.1 Open-Channel Microfluidic Chips

A microchip with a microchannel coating of amine polyethylene glycol has enhanced the separation efficiency of proteins such as bovine serum albumin (BSA), myoglobin, and ribonuclease A and the suppression of non-specific protein adsorption [48]. Cyclic olefin copolymer (COC) microchip effectively reduced the non-specific adsorption of proteins via grafting of *N*-vinylpyrrolidone [27]. The

illustration is achieved by reducing the non-specific adsorption of the model molecule, BSA [49]. A super quick 2D-microchip electrophoresis along with sodium dodecyl sulphate-microcapillary gel electrophoresis (m-CGE) and microemulsion electrokinetic chromatography (m-MEEKC) isolates proteins. Furthermore, this strategy has been utilized to separate nitrosylated proteins from cerebrum tissue of Alzheimer's disease (AD) transgenic mice and epithelial adenocarcinoma cells of the human colon [36].

Similarly, capillary isoelectric focusing (CIEF) chip with m-CGE as a 2D framework working together as a hybrid microchip has been used to isolate proteins. Various proteins, including trypsinogen, BSA, β-lactoglobulin, are utilized to consider the plausibility and productivity. The utilization of low voltage and long run times impedes this microchip [50]. Another manufacture approach consolidating surface functionalization and hot embossing upheld PMMA microchannel, which isolated and preconcentrated proteins utilizing the principle of isotachophoresis (ITP). A protein model of a combination of green fluorescent protein and pacific blue marked human heart troponin I and R-phycoerythrin was utilized for anionic ITP. On the other hand, a protein combination of pacific blue named cardiac troponin I (cTnI) and fluorescein isothiocyanate (FITC) egg whites was utilized in the cationic ITP [51].

4.3.2.2.2 Resin Incorporated Microfluidic Chips

PDMS microchip loaded with mesoporous silica dots of Ia3d space group isolated biomolecules under pressure-driven liquid chromatography. This microfluidic chip proficiently isolated BSA and dextran, a neutral polysaccharide in terms of hydrophobicity [52].

4.3.2.2.3 Monoliths Incorporated Microfluidic Chips

Ethylenediamine is a feeble anion exchange ligand. When incorporated with a functionalized methacrylate monolith in a PDMS microchip, it isolated biomolecules. Utilizing BSA as a model protein, examining various parameters, including pH of the binding buffer and ionic strength, showed the expansion of ionic strength, which showed a reduced protein binding to the microchip. Under optimized conditions, an acceptable detachment of BSA and ovalbumin is accomplished [53]. An incorporated PMMA microfluidic chip separated and evaluated proteins from the human sera. In this, a thin pre-polymerized film of glycidyl methacrylate (GMA)-co-PEGDA is used to immobilize antibodies such as anti-HSP90 (heat shock protein 90), anti-cytochrome C, anti-carcinoembryonic antigen (CEA) and anti-alpha-fetoprotein (AFP). Spiked human sera determine the selectivity of the microchip in various concentrations. The serum matrix is eliminated by affinity extraction, and then the elution unveiled the spiked proteins on the analysis of on-chip capillary electrophoresis [54].

On-chip electrophoresis protein separation is developed with the help of functionalized quaternary ammonium groups. It consists of a monolith, namely 2-hydroxyethyl methacrylate (HEMA)-*co*-ethylene dimethacrylate (EDMA), embedded in the chip. 2-Methacryloyloxyethyl particle is photopolymerized to

accomplish the anion functionality, and this is performed on the monolith. BSA and trypsin inhibitors are effectively isolated through this method [54].

4.3.2.2.4 Nanowire

A microfluidic chip of forest of Si-nanowires (Si-NW) has been developed for the isolation of biomolecules. Furthermore, the specific partition of target analytes and desalting is included. Consolidation of the forest of Si-NW improved the geometrical surface area of the microchip. Biomolecule isolation is contemplated utilizing target analytes such as haemoglobin and enhanced green fluorescent protein (eGFP) and the ligands, namely anti-haemoglobin and anti-eGFP [55]. The majority of the high throughput productivity and processes demand purification as a pivotal downstream processing operation, which is a bottleneck step contributing up to 90% of the total production cost. Highly efficient purification techniques need to be applied for achieving a substantial yield of proteins and nucleic acids in high grade. The past two decades have seen an upsurge in cutting-edge developments of parallelization and complete mechanization of purification processes into the microminiaturized system. The microchannels hollowed out in the microfluidic chips serve as pathways for injected samples containing nucleic acids, therapeutic proteins and enzymes during multiple in-line purifications in bioanalysis. Nowadays, multiple microchips are coupled in series to clarify, pre-purify, purify and dialyze the sample in a single run, intensifying the process [24].

4.3.3 Solid-Phase Extraction

Solid-phase extraction (SPE) is a versatile domain used alone or often integrated with other processes like a polymerase chain reaction inside a microfluidic device, as depicted in Fig. 4.3. Here, the microchambers moulded from polymers PDMS, PMMA, or silicon wafers consist of a single inlet hole or multiple inlet holes, extraction domain, where the rinsing-elusion phases take place and, the outlet port.

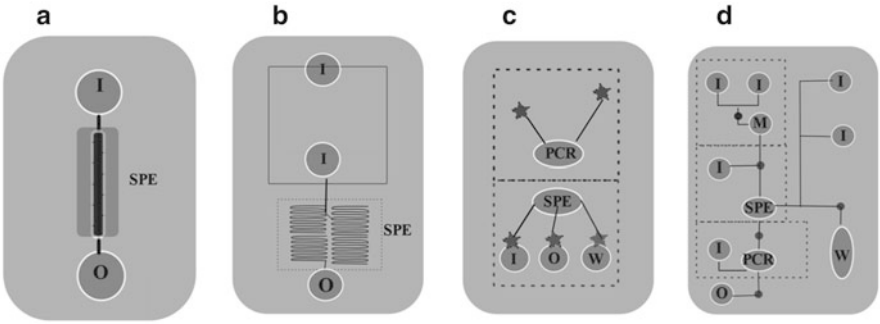

Fig. 4.3 Schematization of chip structures from a simpler to a more complex design. (**a**) Chip with a simple linear structure. (**b**) Chip with a coil-shaped microchannel. (**c**) Chip with electrokinetic motion. (**d**) Chip with a multi-domain design

A single inlet hole is preferred when samples are preloaded with a syringe, while multiple port devices usually have an automatic pump system. The microchip structure may vary according to the sample composition, type and extraction protocol. A typical design has a single microchannel, a T-shaped or convoluted channel (providing a greater surface area and mixing) packed with a capturing material in the form of filtering units, matrices, or functionalized surfaces [56]. The most widely used solid supports are silica based in the form of beads, immobilized particles, micro-posts, micropillars, columns, sol-gels and hybrid sol-gel/silica bead phases. Apart from these, phases that have gained considerable significance are organic polymer monoliths, photoactivated polycarbonates and aluminium oxide membranes [57]. A significant drawback of this approach is the limited surface area available for target analyte adsorption.

Nucleic acid purification is crucial because of the minute target analyte, complex samples, and excessive fragmentation. Any of these factors may introduce contamination in the downstream DNA or RNA, eventually entangling the amplification process, blotting techniques and assays [58]. Notably, this has been overcome, and a high elution yield has been attained by fabricating expendable microfluidic devices using micro-solid-phase extraction (μSPE). The microchambers are designed with chitosan functionalized on microfabricated posts using crosslinker (3-glycidyloxypropyl)trimethoxy silane (GPTMS). Ethanol is passed through the chambers to increase surface wettability and later to wash off excess crosslinkers. A second wash with aqueous NaCl removes excess chitosan. A series of elution buffers mediate the entrapment and release of nucleic acid, so purification is based on the pH value. Thereby, at a low pH value, chitosan interacts electrostatically with negatively charged DNA due to its positive charge (below pK_a 6.5). Higher pH values regain their deprotonated state when the interaction is controlled through buffer exchange [58].

Another typical usage of solid-phase extraction in nucleic acid purification has been demonstrated in convergence with hand-operated microfluidic devices. Here, the implement is multi-layered, that is, a cover layer, thin membrane layer, pneumatic channel and fluidic channel layer. The pneumatic and fluidic layers are fabricated using photolithography, the cover layer by curing PDMS on top of the silicon wafer, and the membrane layer by spin coating a blend of the curing agent and PDMS in the ratio of 1:7. The plasma treatment results in an assembled layer, which is then incubated, and silica microbeads are injected via injection hole and sealed. The device incorporates a reciprocator, switching valves and microfluidic dispenser, which is finger actuated. The valves are operated using buttons in a programmed order. The first button allows the injection of reagents into the microbead column. The second button initiates the agitation of silica microbeads, in turn generating a reciprocating flow. Washing buffer and elution buffer are subsequently added during the process. The technique has been shown to accelerate the recovery rate by increasing the number of washes and agitation and decreasing silica beads' size [59]. These device designs are flexibly applied for the purification of both RNA and DNA.

Another interesting methodology followed for RNA capture and purification is centrifugally controlling the microfluidic device employing solid-phase extraction. The design consists of tetra-ethoxy orthosilicate treated glass microbeads as RNA capture beads forming a matrix with four reservoirs for washing buffer, elution buffer, RNA sample and collecting the sample. The washing and elution reservoirs are joined to microchannel containing bead beds by capillary and siphon valves. The RNA sample, washing buffer and elution buffer loaded into the reservoirs reach the bead bed by revolution per minute (RPM) control. After successfully capturing RNA (a high capture yield), the purified sample is collected in the final reservoir [60].

A combination of electrophoresis and magnetophoresis or solely one of the two can aid purification in microfluidic devices, especially DNA. The chip consists of the following wells: a positive electrode, sample input, elution and negative electrode wells. Solid-phase reversible immobilization magnetic beads coated with carboxyl can selectively adhere the DNA onto its surface and transfer it to the purified buffer upon the action of the external magnetic field. On the other hand, unbound and unwanted DNA molecules (primers and adapters) are driven in the opposing direction by velocity proportional to the external electric field and are removed successfully from the bead cluster. Magnetophoresis alone cannot achieve this velocity by simple diffusion, which is the major drawback of the method. Both magnetic and electric fields simultaneously can migrate the beads from one well to another [61].

4.3.4 Aqueous Two-Phase System

One of the best and most common approaches used for purification and enrichment of proteins and nucleic acids is the aqueous two-phase system (ATPS), also known as the aqueous biphasic system, where water is the primary solvent replacing other organic solvents typically utilized in the conventional liquid-liquid extraction, thus keeping native conformations of analyte intact. The aqueous phases can undergo parallel, slug, or droplet flow patterns depending upon which microchannels are shaped. A Y-shaped inlet (2-inlet) for concurrently injecting two aqueous phases (Fig. 4.4) or a ψ-shaped (3-inlet) microchannel with a third channel for the induction

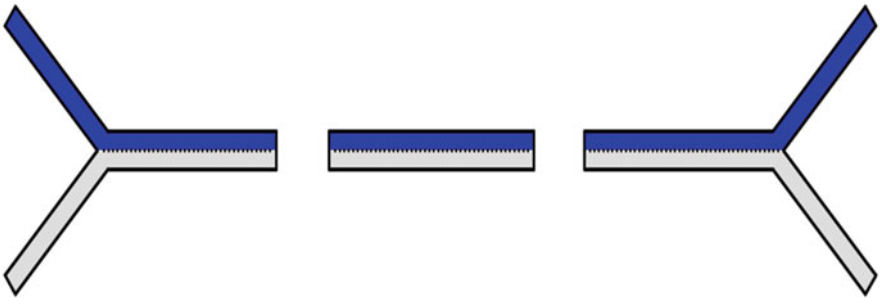

Fig. 4.4 Laminar flow—two inlets and two outlets

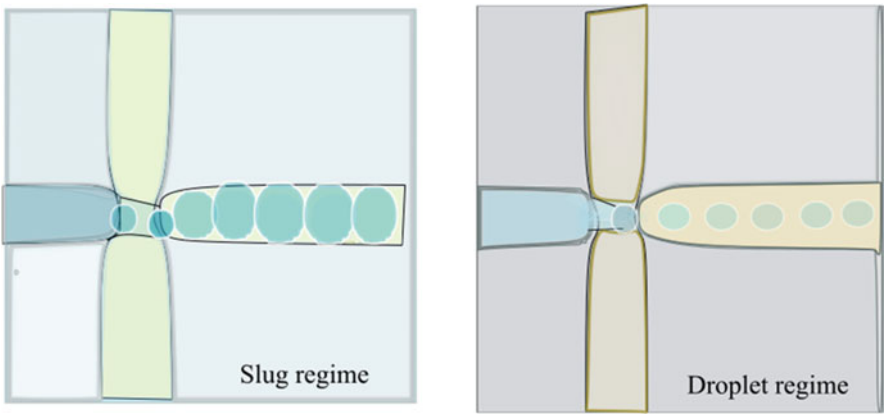

Fig. 4.5 Segmented flow—X-shaped configuration

of a second interface is typical for the laminar parallel flow pattern. Thus, a significant advantage of this pattern is highly efficient mass transfer due to the greater interfacial area and phase separation at the channel exit resulting in the recovery of target analyte-based phase and discard of impurity-based phase. A second conformation is the T shaped or X shaped (Fig. 4.5). Two immiscible phases merge simultaneously, producing instant slugs or droplets due to surface tension and shear forces between the two aqueous phases containing immiscible compounds, causing non-linearity. Here, the surface-to-volume ratio is much higher than the parallel flow. It can also incorporate membranes or micropillars to stabilize co-flowing streams. The two phases maintain a stable interface over the entire length of microchannels due to the interplay of Stokes flow and moderate interfacial tension [62].

Here, the partition occurs due to the preferential interactivity of the analytes and impurities towards respective phases, which sometimes is supplemented by external electric field imposition. Among various two-phase systems, polymer-salt and polymer-polymer are the most conventional aqueous combinations. Recently, polymer-surfactant, surfactant-salt combinations have proven to be successful in protein purification. The polymer-salt is favoured due to its more significant polar gradient between two phases, lower viscosity and faster attainment of thermodynamic equilibrium. These have been markedly applied to purify BSA, bacteriorhodopsin, immunoglobulin G (IgG), green fluorescent protein (GFP), β-galactosidase, protein A, insulin, genomic DNA and several other biomolecules [24]. ATPS is a high-throughput technique on a micro-scale rather than bench-scale liquid-liquid extraction. For instance, PEG/detergent (SDS) ATPS allows successful partitioning of proteins, by complementary solubilization, from impurities. As soon as the crude protein sample enters the central channel of a 3-inlet serpentine microchannel, it is hydrodynamically concentrated by the surrounding flowing streams and the contaminants and the unwanted proteins migrate to the PEG phase. In contrast, the proteins of hydrophobic nature remain in the detergent phase. The flow rate of two

aqueous phases is controlled by microsyringe pumps [63]. The loopholes of inherent partitioning can be circumvented by genetically tagging the desired protein, which can powerfully drive proteins into the PEG phase [62].

4.3.5 Bioinspired Biomolecule Purification

A bioinspired biomolecule purification is a promising approach inspired by naturally functioning biological systems, such as natural sponges and kidneys, to purify proteins such as β2-microglobulin and lysozyme. The design of a serpentine microfluidic channel consists of two different materials, a porous membrane that separates two channels, that is, a sample channel from the dialysate channel, and the heterostructured nanoporous particles fixed at the bottom of the dialysate channel. The former functions as the filtering module for the sample liquid in the sample channel, and the latter is an active sorbent, both clearing out the macromolecules of any impurities. The porous membrane is usually super hydrophilic, sorbent and amphiphilic, such as polyamide membrane and polyacrylic acid-polystyrene divinylbenzene nanoporous particles. The sorbent interacts with the impurities through electrostatic, hydrophilic and hydrophobic forces [64].

4.3.6 Miniaturized Free-Interface Diffusion Devices

Hansen et al. developed the first microfluidic device for high-throughput screening of protein crystallization settings by miniaturizing and parallelizing the free-interface diffusion (FID) approach. This glass or the PDMS system includes valves and pumps (up to 480 valves in the current version) and can perform 144 crystallization experiments on the same chip in 25 nL reaction chambers. Since PDMS is gas permeable, it is possible to prime all of the wells with the help of only a few microlitres of the protein sample by manually pipetting different crystallizing agents. When the valves are opened, FID commences with well-defined interfaces in all of the chambers. The crystallizer must be small since molecular diffusion is the only way to mix the crystallizing chemicals and proteins. There are no buoyancy-driven convection instabilities at such small length scales that would cause unwanted mixing and disrupt crystal formation. Furthermore, lithography allows for accurate construction of the crystallization chamber geometries, allowing for experimentation with mixing kinetics without affecting the final state of equilibrium [65].

4.3.7 Droplet-Based Microfluidics

Ismagilov et al. proposed employing two-phase flow microfluidics to screen crystallization conditions of proteins. These devices take advantage of an inert oil stream's capacity to create aqueous nanolitre-sized droplets [66]. These droplets, which contain specific concentrations of crystallizing chemicals and proteins, operate as

miniature crystallizers with precise volume control (from about 0.1 to 100 nL) [67, 68]. The flow rates of numerous aqueous streams (protein, crystallizing chemicals) are continually altered before the droplets are formed. Using a low-frequency droplet formation (2 Hz for 7.5 nL droplets), they can control the concentrations within a 15% level of certainty. Once flow rates are stopped, various droplets with different crystallization conditions are created, which can be incubated either in the PDMS chip or in an external glass capillary. These approaches can be used to investigate various circumstances utilizing tiny amounts of eaten proteins (a few litres) [69]. The type of oil is a critical consideration in such research. For lengthy periods, per fluoroalkanes oils (PFP) prevent water from exiting the droplets (up to a few weeks), making the droplets ideal micro-batch reactors [68]. However, vapour-diffusion tests replicate water-permeable oils. Researchers held arrays of droplets with different compositions and varying chemical potential differences, which concentrates the protein plugs [70].

Later, they presented a more straightforward technique based on stored droplet arrays. Several 15 nL plugs holding various aqueous reactants are manually made in PFP and stored in a polytetrafluroethylene (PTFE) tube. Gas plugs divide these droplets to prevent coalescence and water permeation between them. When put into a glass capillary, this array can be preserved for months. The array is initially kept in a PTFE tube and subsequently inserted into a PDMS T-junction to screen crystallization conditions. This chip allows the array to be combined with a protein stream flowing at a set rate. In the settings examined by these authors, the protein stream combines within the array's distinct plugs, and the resultant droplets are collected in a glass capillary for incubation. Using only 1 L of a model protein, these researchers examined 48 distinct crystallization settings offered by a commercial sparse matrix [70].

When crystallization hits are discovered through random screening, the goal is to optimize the crystallization by making minor adjustments to the circumstances. Zheng et al. suggested a hybrid method that included the ideas from the earlier works to accomplish simultaneous screening and optimization in nanolitre droplets [67]. The goal is to store an array of big drops (100–150 nL) containing various precipitants [70]. To avoid coalescence and pollution, all of the droplets get separated once more with the help of gas plugs. After that, the array is placed in a PDMS chip and flows under pressure. When paired with a protein and a buffer stream, the big plugs create lengthy segments that act as continuous streams, forming smaller droplets (15 nL) whose composition may be altered by continuously changing the flow rates. For incubation, these plugs are placed in a PTFE tube once more. Zheng et al. used a hybrid approach to accomplish as much as 1300 crystallization trials in 10 nL plugs using only 10 mL of sample, demonstrating that this technology is acceptable for handling membrane protein solutions. However, this approach necessitates a high level of control over the microfluidic characteristics of the droplet fluxes. To begin, a PDMS chip stores droplet arrays and manipulates them as lengthy streams. Second, to adjust the composition, constant modifications in flow rates must be implemented. Because thousands of distinct reactors can be produced in less than 20 min, indexing the droplets is critical. In Zheng's work, droplet indexing is accomplished by continuously adjusting the flow rate of the carrier fluid, which

results in a continuous change in their volume. Finally, to avoid evaporation during long-term storage in the PDMS chip, the input array and output droplets are held in long PTFE tubes [67, 70].

There are a few issues to consider while dealing with droplets. First, the PDMS device elasticity and injection systems frequently cause extended transients with pressure-driven flows, resulting in the consumption of significant amounts of samples (several litres) before stable droplet flows (to know the concentrations, this stability is required). However, more importantly, problems frequently arise when it comes to the mechanics of droplet production. Indeed, it necessitates excellent surface chemistry control and is highly dependent on various factors, such as viscosities, channel geometries and surface tensions. Even while recent research has captured the underlying principles of droplet formation, a thorough knowledge of droplet formation remains elusive. It may thus be difficult for a group lacking a solid understanding of two-phase flows and microfluidic expertise to form droplets, manage their traffic and merge them, mainly when dealing with membrane protein solutions or solutions with a wide range of characteristics (surface tensions, viscosity etc.). Ismagilov et al. contributed significantly to droplet microfluidics and the chemistry of the surfactants and oils employed in the studies. Indeed, they found fluorinated oils that were practically impervious to water and created oligoethylene glycol-capped perfluorinated surfactants that hindered protein adsorption at the droplet interface to replicate perfect micro-batch conditions. They also employed perfluoroamines, which have a high surface tension, to address membrane proteins.

4.3.8 Engineered Micro-batch Experiments

Another method is to use lithography techniques to microfabricate micro-batch. Juárez et al. created a ten by ten array of 5 μL volume-per-element device etched in a silicon wafer early on. Because silicon has excellent thermal conductivity and linear temperature gradients (usually between 15 and 35 °C), the array of wells is silicon coated. With only 250 μL of protein solution pipetted into the wells manually, a microfabricated plate can screen for different temperature settings resulting in diverse nucleation and growth mechanisms. The fluids are not handled microfluidically, and microfabrication is solely employed to create precise geometries in specific substrates [71]. Zhou et al. recently presented a microfluidic solution for fluid handling, which does not require the usage of syringes or valves. The authors microfabricate a well-array (usually 150 wells of 20 nL) in PDMS, glass, or PMMA on a variety of substrates (poly(methyl methacrylate)). PDMS microchannels are constructed and oriented above these wells to prime them [72]. When PDMS is previously degassed, as recommended by Hansen et al., aqueous solutions plugged at the device's inlets spontaneously fill the chambers. Only 5 nL of protein sample fill all of the wells. Now, without using any syringes or valves, an array of crystallization conditions is constructed by aligning chambers filled with different precipitants using the same way. Even while the valves provide for more refined control of the mixing kinetics, mixing between precipitants and proteins happens via an accessible interface diffusion (FID) pathway, as in the

device of Hansen et al. [65]. Evaporation is vital in the above chips since extensive incubation durations are frequently necessary. Evaporation is prevented for months by layers of paraffin oil covering wells engraved on glass slides to overcome these challenges.

4.3.9 Determination of the Microfluidic Phase Diagram to Optimize Crystallization Conditions

In general, crystallization hits are found by searching for reactants that crystallize other proteins at random. The crystals' quality must then be improved by optimizing the conditions. Hansen et al. proposed a microfluidic method that consists of roughly three steps to justify such a strategy [73]. First, large-scale screening of a protein's solubility is carried out in a parameter space that includes multiple buffers and crystallizing agents, as well as varied protein concentrations. When instantaneous precipitation is detected, this solubility fingerprint reveals promising conditions. The kinetic extent of the metastability limit is determined by this screening, which is also known as super solubility rather than thermodynamic solubility [74]. In a second step, detailed phase diagrams around the favourable circumstances reported before are constructed to determine the target protein's super solubility limit. Conditions slightly below super solubility are suitable for the crystallization of the protein. Crystallization studies are then carried out under these circumstances, utilizing either the microfluidic FID described above or traditional micro-batch or vapour-diffusion techniques.

The creation of a microfluidic formulator has enabled this ambitious plan. Based on the PDMS multilayer technique, this complex device combines a microfluidic multiplexor with a rotary mixer. This chip creates mixtures from 32 reactants (proteins, polymers, salts etc.) in a 5 nL ring at precise known concentrations. After a few seconds, the rotary device mixes the reactants, and image analysis determines whether or not precipitation happens. In one day, about 4000 titration tests may be completed with only 8 L of a protein material. The formulator chip is also exploited to screen phase diagrams around the most promising conditions observed during the solubility fingerprint measurement. Each phase space, which has 72 possible combinations, is completed with only 100 nL of protein sample and aids in defining the metastable region's extent. This method has been used to crystallize a variety of proteins that are notoriously difficult to crystallize. In comparison to the traditional random procedure, this method considerably improves the possibility of producing crystals. It is also worth mentioning the device's high-throughput capabilities, which require less sample (a few litres) for more tests (thousands of titrations per day) [75, 76].

4.3.10 Kinetics-Based Passive and Active Control

Kinetics is critical in obtaining high-quality crystals for X-ray observations. It is especially true in the crystallization of protein, which is prone to metastability due to various polymorphs, habits and other non-equilibrium structures (e.g. clusters, gels) [77]. Microfluidics provides excellent potential for precisely following defined kinetic routes. The primary concern for long-term storage is evaporation, especially in microfluidic systems (droplets, micro-wells). Volume loss and concentration evolution induce incorrect calculation of supersaturations which may derail the whole crystallization process. While creating a crystal is frequently the primary purpose of a study, knowing the exact circumstances is necessary to replicate the crystallization conditions in other systems, such as vapour-batch diffusion. The preferred method to solve the evaporation problem is to promote it rather than prevent it. More precisely, by interacting microsystems with an osmotic bath, it is feasible to control the chemical potential by regulating the exchanges between the microsystem and the bath. As a result, incorporating an exchange membrane into microsystems is a critical challenge for technological advancement. While polymeric membranes are used in many microsystems for dialysis, concentration, filtration and other purposes, the role of a PDMS membrane is of particular relevance because it is the basic material of many microfluidic chips. PDMS, although being hydrophobic, is permeable to gas and liquids such as water. Since the 1960s, it has been widely employed in the industry for pervaporation. Evaporation through PDMS can be a severe drawback for long-term storage in PDMS microsystems devoted to protein crystallization, and humidity around the chip must be regulated. In addition, osmotic regulation across PDMS membranes is applied in various microsystems, with the most advanced combining three modules: formulation, addressing and concentration/dissolution cycles. During a crystallization experiment, osmotic regulation allows for fine control of the kinetic routes followed in the phase diagram [78].

The phase chip, developed by Fraden's group, is used to screen the phase diagram of multi-component aqueous systems efficiently. The two fundamental advances of this droplet-based microsystem are docking droplets into wells and contacting them through a thin layer of oil with a PDMS membrane and an osmotic bath beneath. Dry gas or water at a set chemical potential, adjusted with a dissolved salt (NaCl), flows through this osmotic bath. The authors of the two papers closely examine the device's functioning conditions: they can swell or concentrate droplets with great kinetic control. A typical swelling/shrinkage time is about 1 h. However, it varies depending on the ionic intensity of the bath, which may be quantified using a simple model. These osmotic conditions investigate phase diagrams of aqueous solutions such as polymer and salt in water; the latter system is effectively investigated with homogeneous phases, and liquid-liquid breakdown is seen. The chip is then used to study the crystallization of model proteins with neat kinetic control. In particular, the pathway used in the experiments allows for the formation of many nuclei (high supersaturations). The majority of them are then dissolved by osmotic swelling of the drop to select only a few of them, eventually producing good quality crystals in

terms of X-ray scattering. It is thus conceivable to decouple protein crystal nucleation and growth using an osmotic control [79, 80].

In a large-scale version of FID with valves that control the diffusion process and a membrane that provides osmotic regulation, analogue results based on the osmotic exchange were produced. It crystallizes a variety of proteins and figures out what the best conditions are. Furthermore, the experimental settings alter crystallization tendencies. One of the chip's key advantages is that it directly allows cryoprotectants to protect the crystal from X-ray damage. Lau et al. provide demonstrated membrane-controlled crystallization platform. A formulation stage, a droplet injector and a two-phase storage module are all integrated into this sophisticated device. The formulator estimates the limit of metastability by monitoring the appearance of a precipitate in a 5 nL combination of protein and reagents. This chip also enables the creation of nanolitre droplets from designed mixes and their storage in a storage module. The latter links to a membrane exchange zone that ensures the plug's chemical potential remains constant, unlike earlier device iterations where evaporation was an issue. The key strength is the real-time feedback on precipitation and the additional kinetic tuning of crystallization by active osmotic bath management [81].

Droplet-based crystallization tests are useful for manipulating a kinetic pathway via membrane exchange. The exchange of droplets in a train over the oil separating them is analogous but less effective. It is conceivable to alternate solution droplets crystallizing with droplets of brine utilized as a reservoir to concentrate the protein solution via diffusion over the oil spacer. The latter is crucial, as is the space between droplets, regulating the (slow) maturation process. However, in contrast to membrane exchange, the evolution is unidirectional, and the concentration process is not under active control. Aside from evaporation, droplets are also used in a kinetic process for crystal development known as seeding. It effectively separates the nucleation of seed from its subsequent growth in a separate bath with optimal circumstances. In general, high supersaturation is necessary for seed nucleation, whereas low supersaturation is necessary for orderly development, and the two conditions are frequently incompatible. Ismagilov's group uses this technique to stimulate the production of seeds in highly saturated protein and precipitant mixtures (nucleation conditions) and then transport the droplets into larger ones where the solution is diluted to reach orderly growth conditions [82]. Before lowering the supersaturation, the number of seeds can be optimized by adjusting the incubation time. As a result, the two phases are no longer linked, and problematic proteins can now be crystallized.

Since it incorporates optimized geometries (made by lithography) with small length scales, microfluidics eventually allows passive action on the kinetics through a smooth control of the mass transfers. The microfluidic FID is a good example. The geometry of the connecting chambers can tailor the path taken by the FID on its journey to the ultimate mixed state. In a recent study, researchers created channel networks to mimic counter-diffusion crystallization studies in a microfluidic format. The crystallization conditions gradients are calculated based on the channel geometries. Mixing of the compounds occurs only through molecular diffusion in all of these devices due to the tiny length scales, and the channel network's

architecture allows it to act on the mixing kinetics. Taljera et al. developed an evaporation-based crystallization platform to detect and optimize protein crystallization conditions using similar concepts. Microlitre-sized droplets carrying specified amounts of protein and reactants evaporate through a well-defined geometry channel in this device. The channel geometry allows for the fine control of the evaporation rate and, as a result, the kinetic pathway through the phase diagram. These scientists recently demonstrated that kinetic parameters (nucleation, crystal development) might be calculated from such experiments using kinetic models employing such tight control of evaporation and, therefore, on drying rates [83].

4.3.11 Crystal Harvesting Versus On-Chip X-Ray Diffraction

After obtaining a protein crystal, it must be harvested and mounted for X-ray measurements. Crystals are frequently flash-frozen in liquid nitrogen to reduce X-ray radiation damage and reduce thermal noise that causes undesired background. Crystals are usually immersed in a cryoprotectant solution before freezing to prevent them from shattering during this perilous process. Because the diffraction characteristics of the crystals can be altered throughout the harvesting and cryoprotection processes, these processes may be difficult to control. Since these microfluidic devices are not irrevocably sealed, standard crystal harvesting is often possible in the PDMS chips stated previously. However, in situ X-ray diffraction studies show promise in reducing harvesting disturbances. X-ray analysis may be performed directly on-chip in the PDMS devices created by Hansen et al., as shown recently. Cryoprotectant solutions were diffused into the crystal wells over time, and parts of the PDMS chip holding the crystals were frozen using liquid nitrogen immersion [84].

Crystal harvesting is a priori a highly risky process in droplet-based systems, and in situ data collection appears to be necessary. Zheng et al. show direct X-ray diffraction in the capillaries used to retain the droplets, but they also mention significant X-ray damage due to the lack of cryocooling. The same group is now looking into the advantages and disadvantages of in situ data collection in droplet-based systems. This reference thoroughly discusses critical topics such as capillary material (glass versus PTFE), absorption and diffraction background due to the oil phase, and radiation damages. These authors also mention that X-ray analysis on several droplets containing identical crystals may be useful in overcoming the challenges mentioned above.

4.3.12 Challenges

1. Microfabrication technologies such as high-resolution stereolithography, soft lithography and 3D print processes fabricate microfluidic chips. The majority of chips are for one-time usage only. They require specialized equipment (integrated pumps and valves, mixers, micro-fabricated micro-channels, or chambers

specially designed for multiple arrivals of pressurized gas). On the other hand, microfabrication procedures can be costly and time-consuming, necessitating specialized infrastructure and significant handling.
2. Chip materials now in use, such as silicone, hydrogels, glass and elastomers, are not entirely compatible with all crystallization solvents. For example, the most commonly used substance, PDMS (polydimethylsiloxane), is incompatible with organic solvents like acetone. Furthermore, owing to the permeability of the polymer, the solvent may evaporate.
3. It is difficult to change the arrangement because of the mandated channel network geometry, making it less accessible to non-experts.
4. Liquid qualities such as surface tension and flow viscosity are essential in microfluidic liquid handling. As a result, for each working fluid, system calibration is required. It can become quite problematic when dealing with liquids with high viscosity (i.e. polyethylene glycol) or low surface tension (i.e. detergent used for membrane protein solubilization). It is also the fundamental reason why surfactants are used in so many of today's droplet procedures. Surfactants, on the other hand, can interact with the solute molecules, affecting crystallization.

4.4 Microfluidics in Molecular Self-Assembly

Self-assembly in microfluidic devices is a route taken when it comes to the bottom-up approach in the field of nano- or micro-fabrication. Molecular self-assembly is the property of various individual components to self-form spontaneously into the desired shape. This shape gives the compound its function. In general, molecular self-assembly points to the spatial assemblage of nucleic acids or proteins (folding of proteins). However, polymers are made to fold in three dimensions to obtain the requisite structure [85]. Nanoparticles made from DNA have immense potential for various applications such as drug delivery and as a sensing element in various biosensors. Electrostatic interactions are responsible for the self-assembly of these components. This section explains the strategies for developing self-assembled structures using microfluidic devices.

Five main attributes determine the self-assembly of a system [86]. (1) The components involved in the self-assembly. The individual components interacting may be similar or practically opposing. However, the result of their interaction forms a more stable state than their individual existence. (2) The interaction or reaction during the self-assembly. The interactions are generally Coulomb and van der Waals forces, hydrogen bonds, or other hydrophobic interactions. Self-assemblies have coordination bonds. Apart from chemical bonds, complementarity in the interacting molecules' shapes also plays a critical role in self-assembly. (3) Adjustability of assembled molecules is critical in forming stable and ordered structures. The ability to disrupt bonds and reform new bonds in itself helps in obtaining structures with lower entropy. The ability to reversibly bond would imply that the force trying to disrupt the bond is comparable to the forces keeping them associated. (4) The environment in which the components react is critical. An ideal environment is

required for self-assembly to occur. (5) Ability of the interacting molecules to move. At least one of the interacting molecules should be mobile (although, in most cases, all the interacting molecules are in motion). Forces such as thermal motion, Brownian motion, gravitational force, friction and inertia become relevant to the required motion of the interacting particles. The attributes mentioned above are relevant in the self-assembly of particles as small as molecules to as large as galaxies [87].

Free-flow self-assembly and hydrodynamic co-flowing self-assembly are two main strategies for understanding self-assembly in microfluidic devices. The self-assembly occurs in a free-flowing system with the assistance of capillary interactions. Fluidic force, surface force and molecular force are responsible for the self-assembly process. Hydrodynamic co-flowing can produce three different flows: laminar flow, droplet flow and plug flow [88]. The type of flow depends on the capillary number and water fraction. The laminar flow coerces many structures, such as liposomes [89] and scaffolds [90]. Self-assembly of peptides has been known to man for a long time. Modifying peptides to obtain specific structures, ideal for various scientific techniques, has been performed in bulk systems. The same work can be done on a chip with microlitres of liquids. Toprakcioglu et al. has demonstrated a microdroplet array-based system for self-assembly of peptides [91]. Thousands of water-in-oil droplets determine the aggregation kinetics for proteins associated with Alzheimer's disease. Diphenylalanine was also employed to produce microfibrils using the same droplet-based method. A self-assembled monolayer on PDMS using 1-undecyl-thioacetate-trichlorosilane is developed for protein array experiments [92].

DNA origami is the technique in which many short strands of single-standed DNA (ssDNA) self-assemble to form a two-dimensional or three-dimensional structure. Individual strands of DNA are intelligently designed to form unique pairs with another strand. The sequence of the strands determines the shape of the structure formed by DNA origami. A microfluidic device can be designed so that the process of repeated self-assembly of DNA can be performed. This microfluidic DNA origami 'factory' has reservoirs for DNA, linker ssDNA, sample holder etc. It will also employ fluid flow control components such as valves, pumps, separators and mixers. There will be provision for the control of temperature [93]. Applications of self-assembled DNA origami know no bounds. The nanostructures developed using these DNA strands find application in areas such as biosensing [94], catalysis [95], drug delivery [96], therapeutics [97], molecular motors [98], nanorobots [99] and scaffolds for tissue culture [100].

Self-assembly of polymeric nanoparticles and microparticles is an exciting and ever-evolving field. The application of ternary polymeric particles and spherical Janus in various optical and electronic devices has significantly increased. These particles can be produced with the help of microfluidic devices. Nie et al. demonstrated the creation of Janus particles using a microfluidic device. The parallel flowing immiscible monomers can be emulsified at controlled rates and cured using a UV source (Fig. 4.6) [101]. Latest studies have shown that Y-type microchannel produces Janus particles with significant and sharp interphases [102, 103]. A collaboration of sophisticated techniques such as surface-enhanced Raman scattering and

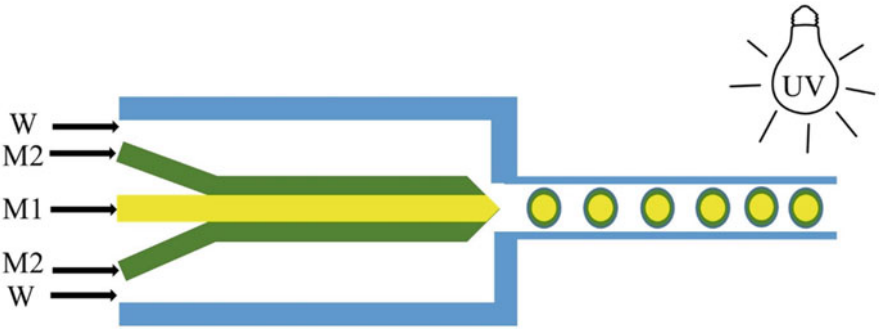

Fig. 4.6 Ternary droplets developed by emulsification of monomers M1 and M2 in aqueous solution of SDS

3D printing has employed Janus particles as a substrate for the quantitative detection of various biomedical analytes [104]. The self-assembly of polymeric nanoparticles does not stop with the Janus. Hydrophobically modified chitosan is used to make nanoparticles with the help of a T-shaped channel. The flow rate and mixing regime is controlled to produce a chitosan-based carrier for the transport of Paclitaxel. Paclitaxel is mixed with the chitosan in the device as a core flow to create drug encapsulated chitosan nanoparticles [105]. Another example of polymeric microparticle self-assembly is the synthesis of the amphiphilic microparticle. The device has a Y-shaped channel wherein the patterns for wedges are embedded. A hydrophilic stream is introduced through one channel, and a hydrophilic stream is introduced through the other channel. The wedge patterns are such that half of the wedge is made with a hydrophobic substance while the other half is a hydrophilic substance. The wedges, due to the positioning of the hydrophobic and hydrophilic layers, self-assemble to form a particle [106].

The latest studies employ self-assembly and microfluidics to their core to produce particles specifically of different applications. Nanoprecipitation using the microfluidic device creates polymeric nanoparticles for drug deliveries [107]. Permanent micromagnets are formed by self-assembly using microfluidics to apply cell sorting [108]. MacFarlane et al. developed π-conjugated polymers for electronic applications [109]. Polymeric self-assembled micelle was developed by microfluidic application for nanoscale drug delivery [110].

4.5 Microfluidics in Chemical Biology: Interesting Applications

Chemical biology and microfluidics hand in hand form an incredible platform of which applications have no bounds. Directly or indirectly, it spans almost all the fields of science. In this section, some of the exciting applications of chemical biology are elaborated.

The flow of micro amounts of fluid is a typical feature of a microfluidic device. These laminar flows are carriers of many components such as proteins, small molecules, growth factors, enzymes and other substances [111]. Flows in microfluidic devices enable the creation of a chemical gradient, exploited for various applications. Microfluidic devices with a continuous gradient of epidermal growth factors, platelet-derived growth factors, and fibroblast growth factor 2 differentiate neural stem cells [112, 113]. The development of cells in a natural environment depends on a gradient of substances such as proteins and small molecules rather than the discrete amounts provided in vitro. It is difficult to mimic these types of concentration gradients on a macroscale. O'Grady et al. developed a platform using hydrogel channels to supply many morphogens to the system [114]. Although this study is at a macroscopic level, it can be easily translated to a microfluidic device [115]. The chemical concentration gradient can be linear or temporal. Lin et al. developed a microfluidic device that can generate different chemical gradients by controlling the flow of solutions entering the mixer [116], giving better flexibility in developing devices that can model various diseases. Microfluidic devices can also be used to provide pH gradients. This has been used to create microfluidic devices for making chitosan membranes [117]. A temperature gradient is yet another characteristic gradient that is important, especially in the field of biology. Shah et al. demonstrated a microwave-based microfluidic device that can give rapid temperature gradients [118].

Advances in fabrication techniques and enhancement of wisdom in chemical biology have led to the creation and development of complicated devices such as the polymerase chain reaction (PCR) on a chip. In 1998, Kopp et al. developed a continuous-flow PCR on a glass microchip with various time zones [119]. Since this demonstration, many researchers have worked tirelessly to develop many improved versions of PCR-on-chip. The improvement may be in terms of engineering or based on its application. Ahrberg et al. broadly categorized PCR as space domain PCR, time-domain PCR, isothermal nucleic acid amplification, digital PCR and commercial PCR [120]. A space domain PCR is a device where the sample is transported through a defined microfluidic channel, wherein the temperature depends on the channel's position in the device. The channels can be serpentine [121, 122], radial [123], oscillatory [124] and heating/cooling systems [125]. A time-domain PCR has a temperature change through heating or cooling while keeping the sample stationary. The heating or cooling can be done by using various tools such as infrared rays [126], lasers [127], or even fans [128]. In isothermal nucleic acid amplification, as the name suggests, the amplification occurs at a constant temperature. Loop-mediated isothermal amplification was demonstrated using multiple primers [129–131]. Digital PCR divides a bulk sample into thousands of sub-samples [132]. The amplified sub-samples have a wide range of applications. It can find specific scarce nucleotides [133]. Thus, digital PCRs detect DNA contaminants in minor quantities [134]. Multiplex PCR is yet another application of digital PCR [135, 136]. Commercial PCR devices are devices with PCR unified into commercial thermal cycles [137, 138]. The research curiosity and advancement in engineering techniques have bought PCRs with improved efficiency and less

complex designs. Devices are further being refined to meet the required applications. Currently, self-propelled continuous-flow PCR [139] and droplet-based digital PCR [140] are developed for various applications.

Single-cell sequencing of DNA and RNA is a promising area with a wide range of applications. The significant outcomes from microfluidics are identifying rare anomalies in cells, studying cell subpopulations, identifying pathogens and studying various diseased cells. Digital microfluidics creates platforms that allow flow based on the design of wells and channels (passively) [141]. Smart-seq2 [142] detects RNA. Zilionis et al. established a fast and efficient method using droplet microfluidics [143]. Hydrogel beads with barcoding primers immobilize the cells. The cells are lysed following which reverse transcription is used to bar code the RNA in it. After this step, all the materials come together once the cells are broken open. Now, cDNA library processing for next-generation sequencing follows. Pellegrino et al. demonstrated a similar single-cell DNA sequencing [144]. Using microfluidic platforms enables comparatively easy sequencing of tens of thousands of individual cells in a matter of hours with very little human intervention. It has direct application in the detection of cancers at very early stages.

4.6 Conclusion

An era is at the horizon where microfluidics is inevitable to the field of science. Microfluidics has found applications in nanotechnology, medicine, chemistry, material science and physics. The use of microfluidics has bought about a system where devices are fabricated with significant dexterity. It has enabled the development of user-friendly systems that put the life of researchers at ease. Microfluidics in chemical biology is proliferating as it requires only minute quantities of samples and reagents, which increases the device's functionality. Even though microfluidics in chemical biology is a significant asset, it comes with its challenges. A major drawback in using microfluidics in chemical biology is the adsorption of material onto the channel walls. Since the surface area of the wall is notably more considerable, the possibility of adsorption is more. Research is happening at a solid pace to develop novel materials to circumvent this issue. Another critical disadvantage in using microfluidics is fouling. Fouling can reduce or even completely block the flow in a channel. Studies are conducted in designing devices that evade this issue. Even though there are disadvantages to using microfluidics for chemical biology, they are belittled regarding the advantages these devices provide. With the motivated and robust research in the field, the cons will be addressed and microfluidics will be the future in the sciences.

References

1. Hou X, Zhang YS, Santiago GT-d, Alvarez MM, Ribas J, Jonas SJ, Weiss PS, Andrews AM, Aizenberg J, Khademhosseini A (2017) Interplay between materials and microfluidics. Nat Rev Mater 2(5):17016. https://doi.org/10.1038/natrevmats.2017.16
2. Niculescu A-G, Chircov C, Bîrcă AC, Grumezescu AM (2021) Fabrication and applications of microfluidic devices: a review. Int J Mol Sci 22(4):2011. https://doi.org/10.3390/ijms22042011
3. Ohno K-i, Tachikawa K, Manz A (2008) Microfluidics: applications for analytical purposes in chemistry and biochemistry. Electrophoresis 29(22):4443–4453. https://doi.org/10.1002/elps.200800121
4. ChipShop m Microfluidics (2021) microfluidic ChipShop. https://www.microfluidic-chipshop.com/
5. Solutions PE Micro Devices Facility (2021). https://www.engineeringsolutions.philips.com/looking-expertise/mems-micro-devices/micro-devices-facility/
6. Soares RRG, Neumann F, Caneira CRF, Madaboosi N, Ciftci S, Hernández-Neuta I, Pinto IF, Santos DR, Chu V, Russom A, Conde JP, Nilsson M (2019) Silica bead-based microfluidic device with integrated photodiodes for the rapid capture and detection of rolling circle amplification products in the femtomolar range. Biosens Bioelectron 128:68–75. https://doi.org/10.1016/j.bios.2018.12.004
7. Reinholt SJ, Craighead HG (2018) Microfluidic device for aptamer-based cancer cell capture and genetic mutation detection. Anal Chem 90(4):2601–2608. https://doi.org/10.1021/acs.analchem.7b04120
8. Yang Y, Rho HS, Stevens M, Tibbe AGJ, Gardeniers H, Terstappen LWMM (2015) Microfluidic device for DNA amplification of single cancer cells isolated from whole blood by self-seeding microwells. Lab Chip 15(22):4331–4337. https://doi.org/10.1039/C5LC00816F
9. Kim SC, Jalal UM, Im SB, Ko S, Shim JS (2017) A smartphone-based optical platform for colorimetric analysis of microfluidic device. Sensors Actuators B Chem 239:52–59. https://doi.org/10.1016/j.snb.2016.07.159
10. Charbaji A, Heidari-Bafroui H, Anagnostopoulos C, Faghri M (2021) A new paper-based microfluidic device for improved detection of nitrate in water. Sensors 21(1):102. https://doi.org/10.3390/s21010102
11. Koesdjojo MT, Pengpumkiat S, Wu Y, Boonloed A, Huynh D, Remcho TP, Remcho VT (2015) Cost effective paper-based colorimetric microfluidic devices and mobile phone camera readers for the classroom. J Chem Educ 92(4):737–741. https://doi.org/10.1021/ed500401d
12. Sechi D, Greer B, Johnson J, Hashemi N (2013) Three-dimensional paper-based microfluidic device for assays of protein and glucose in urine. Anal Chem 85(22):10733–10737. https://doi.org/10.1021/ac4014868
13. Xiao G, He J, Qiao Y, Wang F, Xia Q, Wang X, Yu L, Lu Z, Li C-M (2020) Facile and low-cost fabrication of a thread/paper-based wearable system for simultaneous detection of lactate and pH in human sweat. Adv Fiber Mater 2(5):265–278. https://doi.org/10.1007/s42765-020-00046-8
14. Kumar A, Hens A, Arun RK, Chatterjee M, Mahato K, Layek K, Chanda N (2015) A paper based microfluidic device for easy detection of uric acid using positively charged gold nanoparticles. Analyst 140(6):1817–1821. https://doi.org/10.1039/C4AN02333A
15. Cai L, Wu Y, Xu C, Chen Z (2013) A simple paper-based microfluidic device for the determination of the total amino acid content in a tea leaf extract. J Chem Educ 90(2):232–234. https://doi.org/10.1021/ed300385j
16. Suresh V, Qunya O, Kanta BL, Yuh LY, Chong KSL (2018) Non-invasive paper-based microfluidic device for ultra-low detection of urea through enzyme catalysis. R Soc Open Sci 5(3):171980. https://doi.org/10.1098/rsos.171980

17. Fava EL, Martimiano do Prado T, Almeida Silva T, Cruz de Moraes F, Censi Faria R, Fatibello-Filho O (2020) New disposable electrochemical paper-based microfluidic device with multiplexed electrodes for biomarkers determination in urine sample. Electroanalysis 32(5):1075–1083. https://doi.org/10.1002/elan.201900641
18. Guo X, Guo Y, Liu W, Chen Y, Chu W (2019) Fabrication of paper-based microfluidic device by recycling foamed plastic and the application for multiplexed measurement of biomarkers. Spectrochim Acta A Mol Biomol Spectrosc 223:117341. https://doi.org/10.1016/j.saa.2019.117341
19. Choobbari ML, Rad MB, Jahanshahi A, Ghourchian H (2020) A sample volume independent paper microfluidic device for quantifying glucose in real human plasma. Microfluid Nanofluid 24(9):74. https://doi.org/10.1007/s10404-020-02382-y
20. Noiphung J, Songjaroen T, Dungchai W, Henry CS, Chailapakul O, Laiwattanapaisal W (2013) Electrochemical detection of glucose from whole blood using paper-based microfluidic devices. Anal Chim Acta 788:39–45. https://doi.org/10.1016/j.aca.2013.06.021
21. Rossini EL, Milani MI, Lima LS, Pezza HR (2021) Paper microfluidic device using carbon dots to detect glucose and lactate in saliva samples. Spectrochim Acta A Mol Biomol Spectrosc 248:119285. https://doi.org/10.1016/j.saa.2020.119285
22. Ellerbee AK, Phillips ST, Siegel AC, Mirica KA, Martinez AW, Striehl P, Jain N, Prentiss M, Whitesides GM (2009) Quantifying colorimetric assays in paper-based microfluidic devices by measuring the transmission of light through paper. Anal Chem 81(20):8447–8452. https://doi.org/10.1021/ac901307q
23. Dungchai W, Chailapakul O, Henry CS (2010) Use of multiple colorimetric indicators for paper-based microfluidic devices. Anal Chim Acta 674(2):227–233. https://doi.org/10.1016/j.aca.2010.06.019
24. Vicente FA, Plazl I, Ventura SPM, Žnidaršič-Plazl P (2020) Separation and purification of biomacromolecules based on microfluidics. Green Chem 22(14):4391–4410. https://doi.org/10.1039/C9GC04362D
25. Ou X, Chen P, Huang X, Li S, Liu B-F (2020) Microfluidic chip electrophoresis for biochemical analysis. J Sep Sci 43(1):258–270. https://doi.org/10.1002/jssc.201900758
26. Bao B, Wang Z, Thushara D, Liyanage A, Gunawardena S, Yang Z, Zhao S (2021) Recent advances in microfluidics-based chromatography—a mini review. Separations 8(1):3. https://doi.org/10.3390/separations8010003
27. Fleminger G, Solomon B, Wolf T, Hadas E (1990) Effect of polyethylene glycol on the non-specific adsorption of proteins to Eupergit C and agarose. J Chromatogr A 510:271–279. https://doi.org/10.1016/S0021-9673(01)93761-6
28. Roman GT, Carroll S, McDaniel K, Culbertson CT (2006) Micellar electrokinetic chromatography of fluorescently labeled proteins on poly(dimethylsiloxane)-based microchips. Electrophoresis 27(14):2933–2939. https://doi.org/10.1002/elps.200500795
29. Liang Y, Cong Y, Liang Z, Zhang L, Zhang Y (2009) Microchip isoelectric focusing with monolithic immobilized pH gradient materials for proteins separation. Electrophoresis 30(23):4034–4039. https://doi.org/10.1002/elps.200900209
30. Marshall LA, Rogacs A, Meinhart CD, Santiago JG (2014) An injection molded microchip for nucleic acid purification from 25 microliter samples using isotachophoresis. J Chromatogr A 1331:139–142. https://doi.org/10.1016/j.chroma.2014.01.036
31. Wang R, Xie H, Xu Y-b, Jia Z-p, Meng X-d, Zhang J-h, Ma J, Wang J, Wang X-h (2012) Study on detection of mutation DNA fragment in gastric cancer by restriction endonuclease fingerprinting with capillary electrophoresis. Biomed Chromatogr 26(3):393–399. https://doi.org/10.1002/bmc.1673
32. Henley WH, Ramsey JM (2012) High electric field strength two-dimensional peptide separations using a microfluidic device. Electrophoresis 33(17):2718–2724. https://doi.org/10.1002/elps.201200069
33. Salafi T, Zeming KK, Zhang Y (2017) Advancements in microfluidics for nanoparticle separation. Lab Chip 17(1):11–33. https://doi.org/10.1039/C6LC01045H

34. Li Y, Feng X, Du W, Li Y, Liu B-F (2013) Ultrahigh-throughput approach for analyzing single-cell genomic damage with an agarose-based microfluidic comet array. Anal Chem 85(8):4066–4073. https://doi.org/10.1021/ac4000893
35. Duarte GRM, Price CW, Augustine BH, Carrilho E, Landers JP (2011) Dynamic solid phase DNA extraction and PCR amplification in polyester-toner based microchip. Anal Chem 83(13):5182–5189. https://doi.org/10.1021/ac200292m
36. Tetala KKR, Vijayalakshmi MA (2016) A review on recent developments for biomolecule separation at analytical scale using microfluidic devices. Anal Chim Acta 906:7–21. https://doi.org/10.1016/j.aca.2015.11.037
37. Gubala V, Siegrist J, Monaghan R, O'Reilly B, Gandhiraman RP, Daniels S, Williams DE, Ducrée J (2013) Simple approach to study biomolecule adsorption in polymeric microfluidic channels. Anal Chim Acta 760:75–82. https://doi.org/10.1016/j.aca.2012.11.030
38. Jang Y-C, Jha SK, Chand R, Islam K, Kim Y-S (2011) Capillary electrophoresis microchip for direct amperometric detection of DNA fragments. Electrophoresis 32(8):913–919. https://doi.org/10.1002/elps.201000697
39. Osiri JK, Shadpour H, Soper SA (2010) Ultra-fast two-dimensional microchip electrophoresis using SDS μ-CGE and microemulsion electrokinetic chromatography for protein separations. Anal Bioanal Chem 398(1):489–498. https://doi.org/10.1007/s00216-010-3914-2
40. Zhang L, Rafei SM, Xie L, Chew MB-R, Ji HM, Chen Y, Rajoo R, Ong K-L, Tan R, Lau S-H, Chow VTK, Heng C-K, Teo K-H, Kang TG (2011b) A self-contained disposable cartridge microsystem for dengue viral ribonucleic acid extraction. Sensors Actuators B Chem 160(1):1557–1564. https://doi.org/10.1016/j.snb.2011.08.076
41. Hughes-Chinkhota CN, Banda M, Smolinski JM, Thomas RA, Petibone DM, Tucker JD, Auner GW (2011) Oligonucleotide immobilization using 10-(carbomethoxy)decyl-dimethylchlorosilane for mRNA isolation and cDNA synthesis on a microfluidic chip. Sensors Actuators B Chem 155(2):437–445. https://doi.org/10.1016/j.snb.2010.12.043
42. Viefhues M, Regtmeier J, Anselmetti D (2013) Fast and continuous-flow separation of DNA-complexes and topological DNA variants in microfluidic chip format. Analyst 138(1):186–196. https://doi.org/10.1039/C2AN36056J
43. Kim J, Hilton JP, Yang K-A, Pei R, Stojanovic M, Lin Q (2013) Nucleic acid isolation and enrichment on a microchip. Sensors Actuators A Phys 195:183–190. https://doi.org/10.1016/j.sna.2012.07.022
44. Reedy CR, Bienvenue JM, Coletta L, Strachan BC, Bhatri N, Greenspoon S, Landers JP (2010) Volume reduction solid phase extraction of DNA from dilute, large-volume biological samples. Forensic Sci Int Genet 4(3):206–212. https://doi.org/10.1016/j.fsigen.2009.09.005
45. Kashkary L, Kemp C, Shaw KJ, Greenway GM, Haswell SJ (2012) Improved DNA extraction efficiency from low level cell numbers using a silica monolith based micro fluidic device. Anal Chim Acta 750:127–131. https://doi.org/10.1016/j.aca.2012.05.019
46. Shaw KJ, Joyce DA, Docker PT, Dyer CE, Greenway GM, Greenman J, Haswell SJ (2011) Development of a real-world direct interface for integrated DNA extraction and amplification in a microfluidic device. Lab Chip 11(3):443–448. https://doi.org/10.1039/C0LC00346H
47. Rahong S, Yasui T, Yanagida T, Nagashima K, Kanai M, Klamchuen A, Meng G, He Y, Zhuge F, Kaji N, Kawai T, Baba Y (2014) Ultrafast and wide range analysis of DNA molecules using rigid network structure of solid nanowires. Sci Rep 4(1):5252. https://doi.org/10.1038/srep05252
48. Kitagawa F, Kubota K, Sueyoshi K, Otsuka K (2010) One-step preparation of amino-PEG modified poly(methyl methacrylate) microchips for electrophoretic separation of biomolecules. J Pharm Biomed Anal 53(5):1272–1277. https://doi.org/10.1016/j.jpba.2010.07.008
49. Roy S, Das T, Yue CY (2013) High performance of cyclic olefin copolymer-based capillary electrophoretic chips. ACS Appl Mater Interfaces 5(12):5683–5689. https://doi.org/10.1021/am401081d

50. Lu JJ, Wang S, Li G, Wang W, Pu Q, Liu S (2012) Chip-capillary hybrid device for automated transfer of sample preseparated by capillary isoelectric focusing to parallel capillary gel electrophoresis for two-dimensional protein separation. Anal Chem 84(16):7001–7007. https://doi.org/10.1021/ac3017168
51. Jubery TZ, Hossan MR, Bottenus DR, Ivory CF, Dong W, Dutta P (2012) A new fabrication technique to form complex polymethylmethacrylate microchannel for bioseparation. Biomicrofluidics 6(1):016503. https://doi.org/10.1063/1.3683163
52. Chan AS, Danquah MK, Agyei D, Hartley PG, Zhu Y (2014b) A simple microfluidic chip design for fundamental bioseparation. J Anal Methods Chem 2014:175457. https://doi.org/10.1155/2014/175457
53. Chan AS, Danquah MK, Agyei D, Hartley PG, Zhu Y (2014a) A parametric study of a monolithic microfluidic system for on-chip biomolecular separation. Sep Sci Technol 49(6):854–860. https://doi.org/10.1080/01496395.2013.872144
54. He M, Zeng Y, Jemere AB, Jed Harrison D (2012) Tunable thick polymer coatings for on-chip electrophoretic protein and peptide separation. J Chromatogr A 1241:112–116. https://doi.org/10.1016/j.chroma.2012.04.009
55. Krivitsky V, Hsiung L-C, Lichtenstein A, Brudnik B, Kantaev R, Elnathan R, Pevzner A, Khatchtourints A, Patolsky F (2012) Si nanowires forest-based on-chip biomolecular filtering, separation and preconcentration devices: nanowires do it all. Nano Lett 12(9):4748–4756. https://doi.org/10.1021/nl3021889
56. Obino D, Vassalli M, Franceschi A, Alessandrini A, Facci P, Viti F (2021) An overview on microfluidic systems for nucleic acids extraction from human raw samples. Sensors 21(9):3058. https://doi.org/10.3390/s21093058
57. Wen J, Legendre LA, Bienvenue JM, Landers JP (2008) Purification of nucleic acids in microfluidic devices. Anal Chem 80(17):6472–6479. https://doi.org/10.1021/ac8014998
58. Carvalho J, Diéguez L, Ipatov A, Guerreiro JR, Garrido-Maestu A, Azinheiro S, Prado M (2021) Single-use microfluidic device for purification and concentration of environmental DNA from river water. Talanta 226:122109. https://doi.org/10.1016/j.talanta.2021.122109
59. Park J, Han DH, Hwang S-H, Park J-K (2020) Reciprocating flow-assisted nucleic acid purification using a finger-actuated microfluidic device. Lab Chip 20(18):3346–3353. https://doi.org/10.1039/D0LC00432D
60. Jung JH, Park BH, Choi YK, Seo TS (2013) A microbead-incorporated centrifugal sample pretreatment microdevice. Lab Chip 13(17):3383–3388. https://doi.org/10.1039/C3LC50266J
61. Schneider L, Fraser M, Tripathi A (2021) Integrated magneto–electrophoresis microfluidic chip purification on library preparation device for preimplantation genetic testing for aneuploidy detection. RSC Adv 11(24):14459–14474. https://doi.org/10.1039/D1RA01732B
62. Meagher RJ, Light YK, Singh AK (2008) Rapid, continuous purification of proteins in a microfluidic device using genetically-engineered partition tags. Lab Chip 8(4):527–532. https://doi.org/10.1039/B716462A
63. Hu R, Feng X, Chen P, Fu M, Chen H, Guo L, Liu B-F (2011) Rapid, highly efficient extraction and purification of membrane proteins using a microfluidic continuous-flow based aqueous two-phase system. J Chromatogr A 1218(1):171–177. https://doi.org/10.1016/j.chroma.2010.10.090
64. Fan J-B, Luo J, Luo Z, Song Y, Wang Z, Meng J, Wang B, Zhang S, Zheng Z, Chen X, Wang S (2019) Bioinspired microfluidic device by integrating a porous membrane and heterostructured nanoporous particles for biomolecule cleaning. ACS Nano 13(7):8374–8381. https://doi.org/10.1021/acsnano.9b03918
65. Hansen CL, Skordalakes E, Berger JM, Quake SR (2002) A robust and scalable microfluidic metering method that allows protein crystal growth by free interface diffusion. Proc Natl Acad Sci U S A 99(26):16531. https://doi.org/10.1073/pnas.262485199
66. Li L, Mustafi D, Fu Q, Tereshko V, Chen DL, Tice JD, Ismagilov RF (2006) Nanoliter microfluidic hybrid method for simultaneous screening and optimization validated with

crystallization of membrane proteins. Proc Natl Acad Sci U S A 103(51):19243. https://doi.org/10.1073/pnas.0607502103
67. Zheng B, Roach LS, Ismagilov RF (2003) Screening of protein crystallization conditions on a microfluidic chip using Nanoliter-size droplets. J Am Chem Soc 125(37):11170–11171. https://doi.org/10.1021/ja037166v
68. Zheng B, Tice JD, Roach LS, Ismagilov RF (2004b) A droplet-based, composite PDMS/glass capillary microfluidic system for evaluating protein crystallization conditions by microbatch and vapor-diffusion methods with on-Chip X-ray diffraction. Angew Chem Int Ed 43(19): 2508–2511. https://doi.org/10.1002/anie.200453974
69. Leng J, Salmon J-B (2009) Microfluidic crystallization. Lab Chip 9(1):24–34. https://doi.org/10.1039/B807653G
70. Zheng B, Ismagilov RF (2005) A microfluidic approach for screening submicroliter volumes against multiple reagents by using preformed arrays of nanoliter plugs in a three-phase liquid/liquid/gas flow. Angew Chem Int Ed 44(17):2520–2523. https://doi.org/10.1002/anie.200462857
71. Juárez-Martínez G, Steinmann P, Roszak AW, Isaacs NW, Cooper JM (2002) High-throughput screens for postgenomics: studies of protein crystallization using microsystems technology. Anal Chem 74(14):3505–3510. https://doi.org/10.1021/ac0112519
72. Zhou X, Lau L, Lam WWL, Au SWN, Zheng B (2007) Nanoliter dispensing method by degassed poly(dimethylsiloxane) microchannels and its application in protein crystallization. Anal Chem 79(13):4924–4930. https://doi.org/10.1021/ac070306p
73. Hansen CL, Sommer MOA, Quake SR (2004) Systematic investigation of protein phase behavior with a microfluidic formulator. Proc Natl Acad Sci U S A 101(40):14431. https://doi.org/10.1073/pnas.0405847101
74. Manuel García-Ruiz J (2003) Nucleation of protein crystals. J Struct Biol 142(1):22–31. https://doi.org/10.1016/S1047-8477(03)00035-2
75. Anderson MJ, Hansen CL, Quake SR (2006) Phase knowledge enables rational screens for protein crystallization. Proc Natl Acad Sci U S A 103(45):16746. https://doi.org/10.1073/pnas.0605293103
76. Sommer MOA, Larsen S (2005) Crystallizing proteins on the basis of their precipitation diagram determined using a microfluidic formulator. J Synchrotron Radiat 12(6):779–785
77. Stradner A, Sedgwick H, Cardinaux F, Poon WCK, Egelhaaf SU, Schurtenberger P (2004) Equilibrium cluster formation in concentrated protein solutions and colloids. Nature 432(7016):492–495. https://doi.org/10.1038/nature03109
78. Lounaci M, Rigolet P, Abraham C, Le Berre M, Chen Y (2007) Microfluidic device for protein crystallization under controlled humidity. Microelectron Eng 84(5):1758–1761. https://doi.org/10.1016/j.mee.2007.01.269
79. Shim J-u, Cristobal G, Link DR, Thorsen T, Fraden S (2007a) Using microfluidics to decouple nucleation and growth of protein crystals. Cryst Growth Des 7(11):2192–2194. https://doi.org/10.1021/cg700688f
80. Shim J-u, Cristobal G, Link DR, Thorsen T, Jia Y, Piattelli K, Fraden S (2007b) Control and measurement of the phase behavior of aqueous solutions using microfluidics. J Am Chem Soc 129(28):8825–8835. https://doi.org/10.1021/ja071820f
81. Lau BTC, Baitz CA, Dong XP, Hansen CL (2007) A complete microfluidic screening platform for rational protein crystallization. J Am Chem Soc 129(3):454–455. https://doi.org/10.1021/ja065855b
82. Gerdts CJ, Tereshko V, Yadav MK, Dementieva I, Collart F, Joachimiak A, Stevens RC, Kuhn P, Kossiakoff A, Ismagilov RF (2006) Time-controlled microfluidic seeding in nL-volume droplets to separate nucleation and growth stages of protein crystallization. Angew Chem Int Ed 45(48):8156–8160. https://doi.org/10.1002/anie.200602946
83. Talreja S, Kenis PJA, Zukoski CF (2007) A kinetic model to simulate protein crystal growth in an evaporation-based crystallization platform. Langmuir 23(8):4516–4522. https://doi.org/10.1021/la063734j

84. Hansen CL, Classen S, Berger JM, Quake SR (2006) A microfluidic device for kinetic optimization of protein crystallization and in situ structure determination. J Am Chem Soc 128(10):3142–3143. https://doi.org/10.1021/ja0576637
85. Khoo HS, Lin C, Huang S-H, Tseng F-G (2011) Self-assembly in micro- and nanofluidic devices: a review of recent efforts. Micromachines 2(1):17–48. https://doi.org/10.3390/mi2010017
86. Whitesides GM, Boncheva M (2002) Beyond molecules: self-assembly of mesoscopic and macroscopic components. Proc Natl Acad Sci U S A 99(8):4769. https://doi.org/10.1073/pnas.082065899
87. Isaacs L, Chin DN, Bowden N, Xia Y, Whitesides GM (1999) Self-assembling systems on scales from nanometers to millimeters: design and discovery. In: Perspectives in supramolecular chemistry. Wiley, Hoboken, NJ, pp 1–46. https://doi.org/10.1002/9780470511497.ch1
88. Zheng B, Tice JD, Ismagilov RF (2004a) Formation of droplets of alternating composition in microfluidic channels and applications to indexing of concentrations in droplet-based assays. Anal Chem 76(17):4977–4982. https://doi.org/10.1021/ac0495743
89. Ota S, Yoshizawa S, Takeuchi S (2009) Microfluidic formation of monodisperse, cell-sized, and unilamellar vesicles. Angew Chem Int Ed 48(35):6533–6537. https://doi.org/10.1002/anie.200902182
90. Chung K-y, Mishra NC, Wang C-c, Lin F-h, Lin K-h (2009) Fabricating scaffolds by microfluidics. Biomicrofluidics 3(2):022403. https://doi.org/10.1063/1.3122665
91. Toprakcioglu Z, Challa PK, Levin A, Knowles TPJ (2018) Observation of molecular self-assembly events in massively parallel microdroplet arrays. Lab Chip 18(21):3303–3309. https://doi.org/10.1039/C8LC00862K
92. Hen M, Ronen M, Deitch A, Barbiro-Michaely E, Oren Z, Sukenik CN, Gerber D (2015) An off-the-shelf integrated microfluidic device comprising self-assembled monolayers for protein array experiments. Biomicrofluidics 9(5):054108. https://doi.org/10.1063/1.4930982
93. Huang C, Saeki T, Endo M, Sugiyama H, Weng C, Lee G, Sugano K, Tsuchiya T, Tabata O (2011) Configurable assembly of DNA origami on MEMS by microfluidic device. In: 2011 6th IEEE international conference on nano/micro engineered and molecular systems, 20–23 Feb 2011, pp 197–200. https://doi.org/10.1109/NEMS.2011.6017328
94. Engst CR, Ablay M, Divitini G, Ducati C, Liedl T, Keyser UF (2012) DNA origami nanopores. Nano Lett 12(1):512–517. https://doi.org/10.1021/nl204098n
95. Wang J, Yue L, Li Z, Zhang J, Tian H, Willner I (2019) Active generation of nanoholes in DNA origami scaffolds for programmed catalysis in nanocavities. Nat Commun 10(1):4963. https://doi.org/10.1038/s41467-019-12933-9
96. Zhang Q, Jiang Q, Li N, Dai L, Liu Q, Song L, Wang J, Li Y, Tian J, Ding B, Du Y (2014) DNA origami as an in vivo drug delivery vehicle for cancer therapy. ACS Nano 8(7):6633–6643. https://doi.org/10.1021/nn502058j
97. Kong F, Zhang H, Qu X, Zhang X, Chen D, Ding R, Mäkilä E, Salonen J, Santos HA, Hai M (2016) Gold nanorods, DNA origami, and porous silicon nanoparticle-functionalized biocompatible double emulsion for versatile targeted therapeutics and antibody combination therapy. Adv Mater 28(46):10195–10203. https://doi.org/10.1002/adma.201602763
98. DelRosso NV, Derr ND (2017) Exploiting molecular motors as nanomachines: the mechanisms of de novo and re-engineered cytoskeletal motors. Curr Opin Biotechnol 46:20–26. https://doi.org/10.1016/j.copbio.2016.10.011
99. Li S, Jiang Q, Ding B, Nie G (2019) Anticancer activities of tumor-killing nanorobots. Trends Biotechnol 37(6):573–577. https://doi.org/10.1016/j.tibtech.2019.01.010
100. Bila H, Kurisinkal EE, Bastings MMC (2019) Engineering a stable future for DNA-origami as a biomaterial. Biomater Sci 7(2):532–541. https://doi.org/10.1039/C8BM01249K
101. Nie Z, Li W, Seo M, Xu S, Kumacheva E (2006) Janus and ternary particles generated by microfluidic synthesis: design, synthesis, and self-assembly. J Am Chem Soc 128(29):9408–9412. https://doi.org/10.1021/ja060882n

102. Bai F, Zhang H, Li X, Li F, Joo SW (2021) Generation and dynamics of Janus droplets in shear-thinning fluid flow in a double Y-type microchannel. Micromachines 12(2):149. https://doi.org/10.3390/mi12020149
103. Cheng L, Cai B, Zuo Y, Xiao L, Rao L, He Z, Yang Y, Liu W, Guo S, Zhao X-Z (2017) Janus droplet parallel arrangements using a simple Y-channel flow-focusing microfluidic device. Chem Phys Lett 673:93–98. https://doi.org/10.1016/j.cplett.2017.02.030
104. Litti L, Trivini S, Ferraro D, Reguera J (2021) 3D printed microfluidic device for magnetic trapping and SERS quantitative evaluation of environmental and biomedical analytes. ACS Appl Mater Interfaces 13(29):34752–34761. https://doi.org/10.1021/acsami.1c09771
105. Dashtimoghadam E, Mirzadeh H, Taromi FA, Nyström B (2013) Microfluidic self-assembly of polymeric nanoparticles with tunable compactness for controlled drug delivery. Polymer 54(18):4972–4979. https://doi.org/10.1016/j.polymer.2013.07.022
106. Dendukuri D, Hatton TA, Doyle PS (2007) Synthesis and self-assembly of amphiphilic polymeric microparticles. Langmuir 23(8):4669–4674. https://doi.org/10.1021/la062512i
107. Li W, Chen Q, Baby T, Jin S, Liu Y, Yang G, Zhao C-X (2021a) Insight into drug encapsulation in polymeric nanoparticles using microfluidic nanoprecipitation. Chem Eng Sci 235: 116468. https://doi.org/10.1016/j.ces.2021.116468
108. Descamps L, Audry M-C, Howard J, Mekkaoui S, Albin C, Barthelemy D, Payen L, Garcia J, Laurenceau E, Le Roy D, Deman A-L (2021) Self-assembled permanent micro-magnets in a polymer-based microfluidic device for magnetic cell sorting. Cell 10(7):1734. https://doi.org/10.3390/cells10071734
109. MacFarlane LR, Shaikh H, Garcia-Hernandez JD, Vespa M, Fukui T, Manners I (2021) Functional nanoparticles through π-conjugated polymer self-assembly. Nat Rev Mater 6(1): 7–26. https://doi.org/10.1038/s41578-020-00233-4
110. Ahmadi M, Siavashy S, Ayyoubzadeh SM, Kecili R, Ghorbani-Bidkorbeh F (2021) Controllable synthesis of polymeric micelles by microfluidic platforms for biomedical applications: a systematic review. Iran J Pharm Res 20(2):229–240. https://doi.org/10.22037/ijpr.2021.114226.14769
111. Weibel DB, Whitesides GM (2006) Applications of microfluidics in chemical biology. Curr Opin Chem Biol 10(6):584–591. https://doi.org/10.1016/j.cbpa.2006.10.016
112. Chung BG, Flanagan LA, Rhee SW, Schwartz PH, Lee AP, Monuki ES, Jeon NL (2005) Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. Lab Chip 5(4):401–406. https://doi.org/10.1039/B417651K
113. Kim JH, Sim J, Kim H-J (2018) Neural stem cell differentiation using microfluidic device-generated growth factor gradient. Biomol Ther (Seoul) 26(4):380–388. https://doi.org/10.4062/biomolther.2018.001
114. O'Grady B, Balikov DA, Wang JX, Neal EK, Ou Y-C, Bardhan R, Lippmann ES, Bellan LM (2019) Spatiotemporal control and modeling of morphogen delivery to induce gradient patterning of stem cell differentiation using fluidic channels. Biomater Sci 7(4):1358–1371. https://doi.org/10.1039/C8BM01199K
115. van der Meer AD, van den Berg A (2012) Organs-on-chips: breaking the in vitro impasse. Integr Biol 4(5):461–470. https://doi.org/10.1039/c2ib00176d
116. Lin F, Saadi W, Rhee SW, Wang S-J, Mittal S, Jeon NL (2004) Generation of dynamic temporal and spatial concentration gradients using microfluidic devices. Lab Chip 4(3):164. https://doi.org/10.1039/b313600k
117. Luo X, Berlin DL, Betz J, Payne GF, Bentley WE, Rubloff GW (2010) In situ generation of pH gradients in microfluidic devices for biofabrication of freestanding, semi-permeable chitosan membranes. Lab Chip 10(1):59–65. https://doi.org/10.1039/B916548G
118. Shah JJ, Geist J, Gaitan M (2010) Microwave-induced adjustable nonlinear temperature gradients in microfluidic devices. J Micromech Microeng 20(10):105025. https://doi.org/10.1088/0960-1317/20/10/105025

119. Kopp Martin U, Mello Andrew J, Manz A (1998) Chemical amplification: continuous-flow PCR on a chip. Science 280(5366):1046–1048. https://doi.org/10.1126/science.280.5366.1046
120. Ahrberg CD, Manz A, Chung BG (2016) Polymerase chain reaction in microfluidic devices. Lab Chip 16(20):3866–3884. https://doi.org/10.1039/C6LC00984K
121. Ha ML, Lee NY (2015) Miniaturized polymerase chain reaction device for rapid identification of genetically modified organisms. Food Control 57:238–245. https://doi.org/10.1016/j.foodcont.2015.04.014
122. Jiao Z, Zhao L, Tang C, Shi H, Wang F, Hu B (2019) Droplet-based PCR in a 3D-printed microfluidic chip for miRNA-21 detection. Anal Methods 11(26):3286–3293. https://doi.org/10.1039/C9AY01108K
123. Snodgrass R, Gardner A, Jiang L, Fu C, Cesarman E, Erickson D (2016) KS-detect–validation of solar thermal PCR for the diagnosis of Kaposi's sarcoma using pseudo-biopsy samples. PLoS One 11(1):e0147636
124. Zhang C, Wang H, Xing D (2011a) Multichannel oscillatory-flow multiplex PCR microfluidics for high-throughput and fast detection of foodborne bacterial pathogens. Biomed Microdevices 13(5):885–897. https://doi.org/10.1007/s10544-011-9558-y
125. Chan K, Wong P-Y, Yu P, Hardick J, Wong K-Y, Wilson SA, Wu T, Hui Z, Gaydos C, Wong SS (2016) A rapid and low-cost PCR thermal cycler for infectious disease diagnostics. PLoS One 11(2):e0149150
126. Ouyang Y, Duarte GRM, Poe BL, Riehl PS, dos Santos FM, Martin-Didonet CCG, Carrilho E, Landers JP (2015) A disposable laser print-cut-laminate polyester microchip for multiplexed PCR via infra-red-mediated thermal control. Anal Chim Acta 901:59–67. https://doi.org/10.1016/j.aca.2015.09.042
127. Pak N, Saunders DC, Phaneuf CR, Forest CR (2012) Plug-and-play, infrared, laser-mediated PCR in a microfluidic chip. Biomed Microdevices 14(2):427–433. https://doi.org/10.1007/s10544-011-9619-2
128. Hurth C, Yang J, Barrett M, Brooks C, Nordquist A, Smith S, Zenhausern F (2014) A miniature quantitative PCR device for directly monitoring a sample processing on a microfluidic rapid DNA system. Biomed Microdevices 16(6):905–914. https://doi.org/10.1007/s10544-014-9895-8
129. Giuffrida MC, Zanoli LM, D'Agata R, Finotti A, Gambari R, Spoto G (2015) Isothermal circular-strand-displacement polymerization of DNA and microRNA in digital microfluidic devices. Anal Bioanal Chem 407(6):1533–1543. https://doi.org/10.1007/s00216-014-8405-4
130. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000) Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28(12):e63. https://doi.org/10.1093/nar/28.12.e63
131. Rane TD, Chen L, Zec HC, Wang T-H (2015) Microfluidic continuous flow digital loop-mediated isothermal amplification (LAMP). Lab Chip 15(3):776–782. https://doi.org/10.1039/C4LC01158A
132. Xu G, Si H, Jing F, Sun P, Zhao D, Wu D (2020a) A double-deck self-digitization microfluidic chip for digital PCR. Micromachines 11(12):1025. https://doi.org/10.3390/mi11121025
133. Zhu Y, Lu D, Lira ME, Xu Q, Du Y, Xiong J, Mao M, Chung HC, Zheng G (2016) Droplet digital polymerase chain reaction detection of HER2 amplification in formalin fixed paraffin embedded breast and gastric carcinoma samples. Exp Mol Pathol 100(2):287–293. https://doi.org/10.1016/j.yexmp.2015.11.027
134. Hussain M, Fantuzzo R, Mercorelli S, Cullen C (2016) A direct droplet digital PCR method for quantification of residual DNA in protein drugs produced in yeast cells. J Pharm Biomed Anal 123:128–131. https://doi.org/10.1016/j.jpba.2016.01.050
135. Bian X, Jing F, Li G, Fan X, Jia C, Zhou H, Jin Q, Zhao J (2015) A microfluidic droplet digital PCR for simultaneous detection of pathogenic Escherichia coli O157 and Listeria monocytogenes. Biosens Bioelectron 74:770–777. https://doi.org/10.1016/j.bios.2015.07.016

136. Li Z, Liu J, Wang P, Tao C, Zheng L, Sekine S, Zhuang S, Zhang D, Yamaguchi Y (2021b) Multiplex amplification of target genes of periodontal pathogens in continuous flow PCR microfluidic chip. Lab Chip 21(16):3159–3164. https://doi.org/10.1039/D1LC00457C
137. Bioscience M Flexible molecular platform (2021). https://www.meridianbioscience.com/
138. Hoffmann F (2021) Cobas® Liat® system. Roche Diagnostics International, Switzerland. https://diagnostics.roche.com/global/en/products/instruments/cobas-liat.html
139. Tachibana H, Saito M, Tsuji K, Yamanaka K, Hoa LQ, Tamiya E (2015) Self-propelled continuous-flow PCR in capillary-driven microfluidic device: microfluidic behavior and DNA amplification. Sensors Actuators B Chem 206:303–310. https://doi.org/10.1016/j.snb.2014.09.004
140. Shen J, Zheng J, Li Z, Liu Y, Jing F, Wan X, Yamaguchi Y, Zhuang S (2021) A rapid nucleic acid concentration measurement system with large field of view for a droplet digital PCR microfluidic chip. Lab Chip 21(19):3742–3747. https://doi.org/10.1039/D1LC00532D
141. Xu X, Zhang Q, Song J, Ruan Q, Ruan W, Chen Y, Yang J, Zhang X, Song Y, Zhu Z, Yang C (2020b) A highly sensitive, accurate, and automated single-cell RNA sequencing platform with digital microfluidics. Anal Chem 92(12):8599–8606. https://doi.org/10.1021/acs.analchem.0c01613
142. Picelli S, Faridani OR, Björklund ÅK, Winberg G, Sagasser S, Sandberg R (2014) Full-length RNA-seq from single cells using Smart-seq2. Nat Protoc 9(1):171–181. https://doi.org/10.1038/nprot.2014.006
143. Zilionis R, Nainys J, Veres A, Savova V, Zemmour D, Klein AM, Mazutis L (2017) Single-cell barcoding and sequencing using droplet microfluidics. Nat Protoc 12(1):44–73. https://doi.org/10.1038/nprot.2016.154
144. Pellegrino M, Sciambi A, Treusch S, Durruthy-Durruthy R, Gokhale K, Jacob J, Chen TX, Geis JA, Oldham W, Matthews J, Kantarjian H, Futreal PA, Patel K, Jones KW, Takahashi K, Eastburn DJ (2018) High-throughput single-cell DNA sequencing of acute myeloid leukemia tumors with droplet microfluidics. Genome Res 28(9):1345–1352. https://doi.org/10.1101/gr.232272.117

Role of Microfluidics in Drug Delivery

5

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Abstract

In recent times, there has been rapid improvement and achievement in the development of novel drug delivery systems (NDDS) in a microfluidic environment. Microfluidics technology harnesses the fluid mechanics to generate the delivery systems with unique size and shape that can be used for various pharmaceutical applications. However, the conventional methods require bulky instruments, are expensive, consume more power, have a high thermal loss, and require more time. Further, it is very challenging to automate, integrate, and miniaturize the conventional device on a single platform for synthesizing nanoscale delivery systems. There has been considerable advancement in developing microfluidic devices in the last few decades for NDDS. The microfluidic device unveils several features such as portability, transparency in operation, controllability, and stability with a marginal reaction volume. The microfluidic-based delivery systems allow rapid processing and increased efficiency of the technique by using minimum peripherals for its operation. In this chapter, we have discussed the microfluidic devices used to prepare various formulations for several applications. This chapter summarizes the value chain to develop microfluidic devices, including designs, fabrication techniques, and other related methodologies, to formulate various pharmaceutical drug delivery systems in a controlled and selective manner.

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Keywords

Microfluidics · Novel drug delivery · Microfluidic designs · Pharmaceutical formulations · Soft lithography · T-Junction

5.1 Introduction

Recent advances in drug delivery systems can certainly influence the therapeutic outcome, thereby patient compliance. Due to such progressions, it is not astonishing that the US market for drug delivery systems has exploded since the year 2000. The traditional drug delivery approach is a lengthy process involving a multi-step procedure that necessitates significant resources for chemical characterization, mass production, cell viability, toxicity testing, feasibility, preclinical animal and human trials. Nanoparticles are undetectable, tiny particles that range in size between 1 and 100 nm. There are several potentially promising nanoparticle-based drug delivery technologies in development at the moment, but only a few have made it beyond the clinical trial stage. Recent developments in microfluidics technology have enabled a new way for drug delivery techniques and have shown the potential for facilitating the development of various drug delivery systems [1–3]. Since its introduction in the early 1985s, the microfluidic technology has gained well-proven and exponential growth to process or manipulate the micro/nanovolume of fluids (10^{-6} to 10^{-9} L) within microchannels of 10–100 μm. Microfluidic devices are responsible for fluid sampling, manipulating, transferring, mixing, monitoring, and analyzing [4]. These devices are especially spreading into biological, biomedical, and biochemical research areas revealing significant potential in automation, integration, miniaturization, and cost-effective commercial devices. An important feature of microfluidics is that the selection of the material dominates and determines its function [5, 6]. On the microscale, the surface properties of the materials are substantially enhanced, which can either realize unique purposes or lead to difficulties that would not be encountered at the macroscale. Microfluidics systems find many applications such as blood analysis, DNA sequencing, chemical synthesis, biochemical sensing, protein analysis, inkjet printing, and drug delivery [7–9]. Microfluidic devices can easily be integrated with the development process of various drug delivery systems, sensors, and operators. Further, microfluidics provides numerous advantages over conventional approaches, which are laborious and tedious. Figure 5.1 shows the comparison of the biochemical analysis carried out using conventional and microfluidic approaches.

The formulation of drugs and their delivery system is an essential advancement field toward better health and comfort. The pharmaceutical formulation combines a range of excipients with active pharmaceutical ingredients (API) to create a product that can be successful in-patient administration. Moreover, these formulations are clinically relevant as they improve drug solubility, stability, and bioavailability [10]. The formulation of a drug is an important step in drug development as it

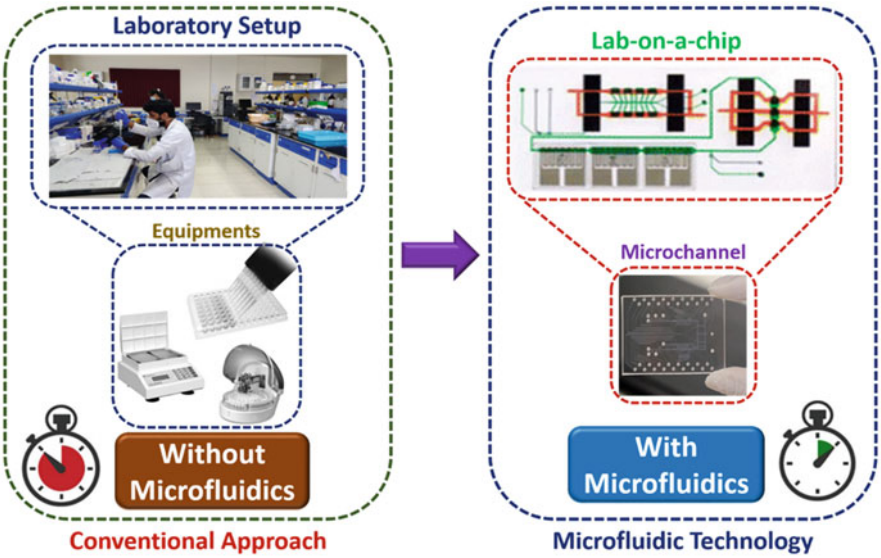

Fig. 5.1 Comparison of various biochemical analyses carried out using conventional and microfluidic approaches

helps deliver the drug to a specific site in the body at optimal concentration. To summarize, formulation development serves various purposes, such as,

- Increases the solubility, stability, and bioavailability of drugs.
- Increases the efficacy and decreases the toxicity of drugs when compared with the free form of the drug.
- Increases patient safety and improves handling properties.

Commonly used formulations for various drugs include tablets, suspensions, capsules, emulsions, ointments, drops, suppositories, and injectables. These formulations have different applications based on their route of administration. Tablets and capsules are very old but commonly used formulations, as they are more feasible and easily available medications than other formulations. Various types of tablet formulations are available in the market, like sustained-release, disintegrant, chewable, and effervescent tablets. Capsules are commonly used for drugs that have a bitter taste. Generally, capsules are used to mask the taste of the particular drug and also protect the drug from the external environment, which may affect the stability of the drug. Other oral dosage forms like suspension and emulsion are available in liquid forms [11].

Developing a suitable formulation to optimize the drug concentration with high efficacy and minimum toxicity has always been a challenge for researchers in academics and industries. Drug development is a time-consuming and expensive process that comes with a significant level of uncertainty about the success of drug

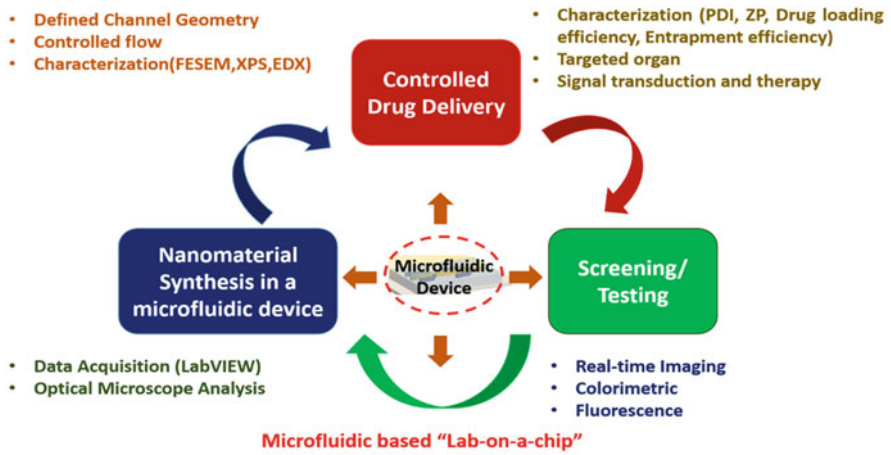

Fig. 5.2 Overview of the microfluidic platform in drug delivery applications

molecules. Moreover, a newly developed drug cannot be administered to patients in its raw form, hence requiring a conventional or novel drug delivery system. A significant amount of time, human resources, and a high cost are involved in developing and monitoring the consequences of the optimal formulation/drug delivery systems via trial-and-error methods. Other challenges commonly faced during the development of drug delivery systems include low solubility and poor permeability of drugs, scalability of formulation, and shelf-life of the product [12].

Many drug delivery techniques have been evolving to overcome the challenges mentioned above. Some recently developed novel drug delivery systems include microparticles and nanoparticles, such as liposomes, micelles, and niosomes. These delivery systems have many advantages over conventional delivery systems as they enhance the dissolution of drugs and are also target-specific. In addition, these novel delivery systems improve the permeability of drugs into the body, prevent the degradation of drugs by hindering their interaction with various enzymes, and increase drug molecules half-life due to their size. In spite of their successful development and clinical usage, the development of these systems still faces challenges such as characterization, cost-effectiveness, multiple processing steps, reproducibility, and scalability. However, progress is ongoing in terms of research and studies, and these systems have shown promise in real-time applications. Figure 5.2 shows the complete overview of the microfluidic platform in drug delivery applications. Real-time characterization techniques are an area for development in existing microfluidic designs.

Revolutions in the fabrication of delivery carriers and the development of direct administration of drugs have been made through microfluidic devices. It provides very stable, uniform, high drug loading, and dispersed carrier particles that can be produced by designing appropriate geometries and selecting correct volumes and fluidic rates of the fluids in the channels of the microfluidic chip for high precision. It

can overcome the drawbacks of conventional drug delivery systems by requiring lower volumes of valuable drugs or chemicals, improving release profile by generating monodisperse particles, generating better drug loading, thereby improving drug delivery localization and minimizing the toxicity [13].

Improved mass transfer reducing mixing time, high surface to volume ratio improving heat exchange properties, precise flow control, deterministic flow, that is, low Reynolds numbers and thus laminar flow, limited reagent volume, continuous regime, ease of manufacture, compatibility with various reagents, costs, and so on are all advantages of microfluidic devices when it comes to drug delivery [14] providing great potential in various applications including drug delivery to the skin, eye, and brain. Moreover, microfluidics has essential characteristics that are highly beneficial for drug delivery, such as enhanced mass transfer, which decreases mixing time, a high surface to volume ratio, which increases heat exchange properties, and accurate flow control deterministic flow [15].

5.2 Design of Various Microfluidic Platforms

Various microfluidic designs can be used to prepare a pharmaceutical formulation, depending on the requirements. They vary by a requirement in terms of materials used for manufacturing and construction of channels, the shape and geometry for different flow rates, external mixers, and various droplet-based devices, and these different configurations produce different results [16].

5.2.1 Materials

In the past few decades, several materials have been harnessed to realize diverse microfluidic devices. It is pertinent that selecting the appropriate material for a microfluidic device is the first and most often crucial step which is the key for various applications [17]. However, to accomplish specific functions, extra attention should be paid to selecting the appropriate material for developing a microfluidic device, as this influences the intrinsic properties and microfabrication techniques. A microfluidic device is a molded or engraved pattern of microchannels with an inlet and outlet for infusing and collecting reaction samples on a fabricated substrate. Microfluidic devices, which may include valves for active flow control, can be easily controlled with the movement of the fluid in the microchannel for various applications [18]. A microfluidic device design network must be customized to satisfy the requirements of the desired applications like pathogen detection, lab-on-a-chip, and electrophoresis to accomplish the required outcome. As a result, the materials used in microfluidic devices must be adequate and should possess the necessary qualities [19].

Since the inception of microfluidic devices for most applications, the widely used original materials were glass and silicon. However, as time progressed, new materials with technological developments emerged in the microfluidic

Table 5.1 Different materials used in realizing microfluidic devices

Materials	Thermal conductivity (W/mK)	Electrical conductivity (Ωm)	Dielectric constant	Maximum temperature (°C)
Polydimethylsiloxane (PDMS)	0.15	4×10^{13}	2.3–2.8	350
Silicon	1.05	6.40×10^{2}	2.81–3.56	315
Glass	0.8	10×10^{10}	5–10	400
Paper	0.05	1×10^{8}	3.7	200
Polymethyl methacrylate (PMMA)	0.1–0.5	10×10^{22} to 10×10^{30}	3–5	120–210

environment, and materials such as paper, polymer substrates, and composites were used to fabricate such devices. Further, materials for microfluidic devices can be classified into three categories: paper, polymeric, and inorganic. In general, the materials to fabricate microfluidic devices have evolved to represent two important microfluidic technology trends: microscale research platforms and low-cost portable analysis devices. These portable analysis devices are helpful in conducting analytical tests in the proximity of the patient or at another isolated location. When choosing materials for laboratory research, chemists usually make a trade-off between prototyping simplicity and device performance [20]. However, the cost of production and the convenience and dependability of usage are the most important considerations when it comes to commercialization.

The major points to keep in mind for manufacturing traditional microfluidic devices are reagent compatibility, optical transparency, conductivity, thermal properties, and easy and mass fabrication [21]. Table 5.1 illustrate different materials used in microfluidic technology for drug delivery applications.

Polydimethylsiloxane (PDMS) and polymethyl methacrylate (PMMA) tend to swell in contact with strong solvents. Swelling is not desirable for uniform fluid flow and carrier generation. It can also lead to deformation or blockage of microchannels. PDMS does not react very well with non-polar solvents, like ethanol and absorbs small ions [22]. Therefore, depending on the requirements, the most appropriate device and solvents need to be chosen. Additional properties like the fluid flow rate, viscosity, Reynolds number, and geometry of microchannels with respect to nanoparticles can also be customized as per our custom requirements [12]. Lithography is a common production method; it prints directly onto suitable material. Apart from lithography, micromachining and injection molding are popular. Micromachining helps to transfer patterns in a resist to the substrate, while injection molding uses thermoplastic materials to make macro/microscopic things [21]. Glass and capillary-based microfluidic devices are used for the generation of emulsions, microcapsules with co or flow-focusing geometry. Further, the microfluidic devices use the parallelization technique to compartmentalize multiple samples and droplets [21]. This reduces the amount of reagent required and reagent device interactions.

Table 5.2 Summary of various fabrication techniques used in a microfluidic platform

Fabrication techniques	Material substrates	Advantages	Disadvantages
Screen printing	Paper, wood, polymer, cloth, glass	Simple, versatile, cost-effective	Consumes more time, slow process, and poor resolution
Photolithography	Silicon, printed circuit board, glass	Better resolution, less wavy patterns	Expensive, requirement of cleanroom facility, ultraviolet (UV) radiation
Soft lithography	Polydimethylsiloxane	Easier setup, relatively low cost, and high throughput	Optical diffraction, not applicable for non-planar surfaces
3D printing	Filament, resin	Flexible design, rapid prototyping, ease of access	Restricted microchannel dimension
Laser ablation	Polymer, polyimide	Allows control over material properties using process parameters, including laser tuning and multi-target precursors	Consumes more power
Hot embossing	Polymer	Low material flow, minimum internal stress	Difficulty in demolding and significant residual thermal stress
Wax printing	Paper	Simple, low-cost, no need of passive components like pumps and valves	Low resistance to heat and toxic reagents

Table 5.2 summarizes the various fabrication techniques used in a microfluidic platform.

5.2.2 Microfluidic Channel Design and Geometry

There are three basic geometries of microchannels or microreactors in microfluidic devices: chamber-based, continuous-flow, and droplet-based microfluidics.

5.2.2.1 Chamber-Based Devices

Microfluidics is both the science of studying the behavior of fluids via microchannels and the technology of producing microscaled devices with chambers and tunnels through which fluid flow is contained. It is simple to manufacture. It may be created from a single layer PDMS microfluidic device. Microfluidic chambers are largely utilized in cell culture research.

5.2.2.2 Continuous-Flow-Based Devices

Continual flow microfluidics is the modification of flow of liquid through manufactured microchannels without disrupting continuity. External sources such as micropumps (e.g., peristaltic or syringe pumps) or other internal processes establish fluid flow.

5.2.2.2.1 T or Y Junction

It is the simplest geometry [22]. Integrated heater devices, magnetic valves, acoustic devices, moving wall structure, high voltages come under active controls. Passive control involves osmosis, diffusion, gravity, pressure differences to control the flow rate. Figure 5.3 shows the different types of design approaches in microfluidic technology.

5.2.2.2.2 Co-flowing Junction

Here a smaller capillary containing the dispersed phase is inserted into a larger diameter channel. The shear force becomes stronger when the dispersed phase enters the larger channel and is surrounded by the continuous phase [22].

5.2.2.2.3 Flow Focusing Junction

It usually has three microchannels. One contains the dispersed phase and is surrounded by two continuous phase channels. After mixing, the solution is focused through a narrow orifice. The geometries can be varied to obtain planar or cylindrical flow patterns.

All the above three simple geometries can be added together to the design to get the desired result. For example, there can be multiple T junctions after another or a T junction followed by co-flow geometry. All configurations give different droplet compositions for drug delivery. Mixers like Herringbone are used for creating platforms for self-assembled drug delivery systems. Carriers are formed at the interfacial layer after the interaction of multiple solvents [22].

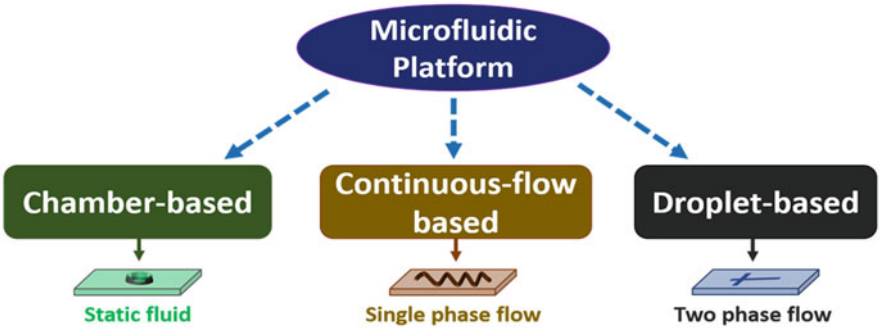

Fig. 5.3 Different types of design approaches in microfluidic technology

5.2.2.3 Droplet-Based Devices

Droplet-based microfluidics is a subset that uses immiscible phases to generate droplets or discrete compartments. Microfluidic devices are distinguished by their low Reynolds number flow, requiring all fluid flow to be essentially laminar. Continuous-flow-based systems have utilized this phenomenon to produce a plethora of unique micro-environments. A substantial number of reactions can be done using droplet microfluidics, yet the device size and complexity are not increased as much. As a result, it was found that microfluidic droplet systems can perform simple Boolean logic tasks, which is a big step toward building a microfluidic computer chip [26]. The most widely utilized carrier production technology is monodispersed and size-controlled nano- and microparticles. This approach allows biomolecules to be encapsulated into discrete droplets and then analyzed using these units. A droplet-based system is the process of manipulation and creation of droplets within microfluidic devices. Heat and mass transfer durations, diffusion distances being short lead to quicker reaction times. This happens due to the large surface area to volume ratios at the microscale. Droplet-based devices, as opposed to continuous-flow systems, allow for autonomous control of each droplet. As a result, microreactors can be moved, combined, and studied independently [27]. Table 5.3 summarizes different designs employed in the development of the microfluidic device.

5.3 Application of Microfluidics in Various Drug Delivery Systems

Commonly nano/novel-drug delivery systems (NDDS) are composed of a large volume of fluids. The conventional manufacturing process poorly controls the physicochemical characteristics of the manufactured NDDS, and also there are batch-to-batch variations. Therefore, it has a significant impact on their applications for drug delivery. As a liquid combination is handled manually at a macroscopic level, it is difficult to manage the molecular assembly required for the formation of a new drug delivery system. In this context, microfluidics can produce a uniform and orderly mixing of different liquids compared to traditional methods. Evidently, the

Table 5.3 Summary of different designs employed in the development of the microfluidic device

Design	Advantages	Disadvantages	References
Y or/and T junction	The linear distance along the channel, linear function of time, constant flow rate	High pressure losses due to small size	[23]
Straight microchannel	Simple approach and easy process of fabrication	Limited for specific use	[16]
Serpentine or/and trapezoidal	Well mixing of samples, any obstruction in the path will not block	A reactant is depleted through the length of the channel	[24, 25]

use of microfluidics resulted in the encapsulation of drugs with a high structural homogeneity and reproducibility level [29].

A unique advantage of microfluidics is the ability to develop effective drug carrier systems and cell-free protein synthesis systems, as well as rapid ways to screen drugs. Comparatively to bulk methods, multiphase fluids can be more effectively controlled by controlling manufactured chip geometries and flow rates. Microfluidics systems produce uniform, stable, and monodispersed particles with high encapsulation. Microfluidic devices should be designed considering several factors, including the type of materials used in the manufacturing process. The following materials are compatible with the different dimensions and types of solvent microchannels, inputs, and mixers. Polydimethylsiloxane (PDMS) and poly (methyl methacrylate) (PMMA) are the most common polymers used in the manufacture of microfluidic devices by gentle lithography [30–32]. By allowing oxygen to penetrate, these materials can be used to construct a device. Several kinds of mixers and synthesizers can be connected to these materials [22]. The descriptions of various types of carriers and their production using microfluidic devices are listed in Table 5.4.

5.3.1 Emulsions

Emulsions are dispersed mediums of solutions and are thermodynamically unstable systems. They are spontaneously formed with the help of surfactants, which improve long-term stability and reduce coalescence between droplets [22]. Emulsions have several applications in food processing, oil recovery, cosmetics, textiles, and pharmaceuticals [33]. A dispersed phase is converged with a continuous phase while mixing two immiscible liquids, leading to a single emulsion. Hydrophobic drugs are usually formed by water-in-oil droplets where oil is the continuous phase. On the other hand, an oil-in-water emulsion is suitable for hydrophilic drugs where water is the continuous phase. Multiple emulsions can be defined as emulsions within emulsions where both oil-in-water and water-in-oil emulsion exists concurrently. Several drops of oil/water are first immersed into the water/oil phase, then into yet another phase. The number of internal phases can be classified as double, triple, and quadruple emulsions. As long as the nature of the phase is similar, water can be replaced by any other aqueous or polar solution and oil by any organic or non-polar liquid. Out of all the types, double emulsions are the most sought after [12]. The size, structure, composition, and production of droplets in double emulsions can be easily tailored and simplified. Moreover, these droplets can also be used as a template for future microcapsules.

5.3.1.1 Issues with the Preparation of Emulsions and Improvement via Microfluidics

The physical characteristics of emulsions vary with pH, mixing rate, temperature, shear force, molecular concentration, and geometry of chosen surfactant. Due to the manual preparation of emulsions, shear stress is generated, which is responsible for

Table 5.4 Types of drug delivery systems prepared using microfluidic techniques

Drug	Type of drug delivery systems	Existing problem	Application of microfluidics	Design	References
Omega-3 fatty acid conjugated taxoid prodrug	Emulsions	Large polydispersity, inconsistent structural integrity and encapsulation issues	Can obtain controllable emulsions for producing uniform particles. It can prevent creaming, increase life span, and can make them thermodynamically stable	Droplet-based microfluidic device and capillary microfluidic devices	[12, 22, 33, 34]
Aspirin	Liposomes	A time-consuming process, improper mixing, uneven particle size and turbid solutions	It reduced the number of steps and time taken to produce monodisperse liposomes. Uniform particle size, efficient mixing and clear solutions	Droplet-based microfluidic device	[27]
Doxorubicin	Nano-liposomes	The size of the formulation cannot be controlled	Size and size distribution can be controlled by flow-rate	Y-type microfluidic device	[35]
Paclitaxel	Micelles	Manual mixing, uncontrollable particle size	Proper mixing can form self-assembling amphiphilic copolymers	T junction	[36]
Topotecan	Niosomes	Large size niosomes with large Polydispersity index (PDI) don't have good reproducibility of encapsulating drugs	The size and size ratio can be adjusted by manipulating the aqueous and organic flow ratio	T-type microfluidic device	[37]
Curcumin	Nanoparticles	Larger size and non-uniformity	By adjusting the flow rate, nanoparticle size, and size distribution, it can be enhanced	Y-type device	[38]
Poly-ethylenimine DNA complexes	Polyionic complex	Lipoplexes have smaller structures with fewer layers	Have the unique capability to control the mix of liquids and allow assembly of polyionic complexes	T junction	[39]
Vitamin E	Nanocapsules	Larger size and don't have reproducibility	The size of fabricated nanocapsules can be adjusted and reproducible. Self-assembly can also be improved using external technologies, like UV, etc.	Y-type devices	[40]

phase inversion and auxiliary/membrane emulsification. These traditional methods lead to the manufacture of highly polydisperse emulsions in bulk quantities but are unable to produce uniform droplets. Moreover, creaming and short half-life of the emulsions are a few of its major disadvantages. In addition, large polydispersity, inconsistent structural integrity, and encapsulation caused by high shear are other disadvantages associated with the conventional preparation of emulsion [33]. Emulsions with uniform and size-controlled particles can be prepared by using suitable microfluidic devices. It is easy to generate single, double, and multiple emulsion features using this technique. Droplet microfluidics can be utilized to prevent the above limitations and obtain controllable emulsions for producing uniform particles. Two main types of preparation methods of emulsions using the microfluidic device are continuous flow-based methods that require immiscible liquids and electrowetting-based methods where tension is manipulated between solid, liquid, and ambient fluid using electrical fields to create a reservoir and breaking off from it to generate droplets [22]. The fabricated device contains the desired channels or fluid paths which can be sealed with another identical block or thermal annealing. The inlet and collection microchannels (drops forming zone) have the same depth. However, the continuous phase cannot encompass the dispersed phase completely because of the rectangular shape of channels. Hydrophilic channels are required for oil-in-water emulsion, and locally modified hydrophobic and hydrophilic channels are required for multiple emulsions [41].

5.3.1.2 Usage of Microfluidic Devices for Emulsion Preparation

5.3.1.2.1 Production of Single Emulsions Using Microfluidics

Microfluidics is now used commonly for the production of emulsions. Depending on the geometry, there are two setups of drop formation: co-flow and flow-focusing. Both these setups consist of coaxial groupings of capillaries made of glass on the microfluidic plane [34].

5.3.1.2.1.1 Co-flow Glass Microfluidic Device

In this example of co-flow setup of drop formation, the outer diameter of the capillary is around 1–2 mm [34]. To design the faucet, a uniform orifice is placed at the end of a tapered geometry generated by heating the ends of the capillary and tapering it using a pipette puller. This capillary is circular and wedged into a square capillary, which constitutes device formation [34]. The inner diameter of the square capillary is matched to the outer diameter of the circular capillary before connecting them. This ensures a coaxial alignment between the two capillaries. Each capillary is responsible for the flow of a single fluid, and as the fluids flow in the same directions, the name is given as coaxial flow/co-flow.

5.3.1.2.1.2 Flow-Focusing Glass Microfluidic Device

In contrast to the co-flow setup, the fluids flow in opposite directions in the flow-focusing setup, generated from the ends of the square capillary. It also contains a narrow orifice and a tapered geometry for the circular capillary. Here, the outer fluid

is responsible for the hydrodynamic flow of the inner fluid. It generates droplets smaller in size as compared to the orifice, which is the major advantage of this setup [34].

5.3.1.2.2 Production of Multiple Emulsions Using Microfluidics

5.3.1.2.2.1 Capillary Based Microfluidic Devices

Double emulsions can be made by integrating both co-flow and flow-focusing techniques of glass capillaries microfluidics setup, as discussed above. The inner diameter of the square capillary and the outer diameter of the circular capillary are matched again for coaxial flow. This device comprises two circular capillaries, one square capillary, and three fluids where the middle fluid is immiscible with the other two [34]. The inner and middle fluid flows in the same directions through the tapered end of one circular capillary and outer capillary, whereas the outermost fluid flows in the opposite direction to hydrodynamically oppose the coaxial stream of other liquids flowing in another direction.

These three fluids then proceed towards the collection tube to form a double emulsion [34]. The outermost fluid can also be introduced stepwise to the co-flowing stream for improved flexibility. Thickness, size, and distribution of shell and various stimuli-responsive elements can control emulsions, encapsulation and release kinetics. Figure 5.4 shows the capillary flow approach in a microfluidic device.

5.3.1.2.2.2 Planar Microfluidic Devices

1. Two drop makers: Planar microfluidic device can be constructed using two drop makers with varying surface wettability [42]. It is a two-step process where the second drop maker is responsible for adding the middle phase to envelope the inner drops made by the first drop maker, thus creating double emulsions. The device can have multiple configurations such as two T junctions, two cross junctions, or a T junction followed by a flow-focusing unit.
2. Double flow-focusing: A device comprising double flow-focusing units can be fabricated. There are central and side microchannels introducing the continuous aqueous fluid (inner fluid) and the other two liquids (middle and outer fluid), respectively, as shown in Fig. 5.5. A compound jet is used to form core/shell drops, where the instability of the jet leads to the formation of drops in the downstream chamber after the inner and middle fluid is focused through an

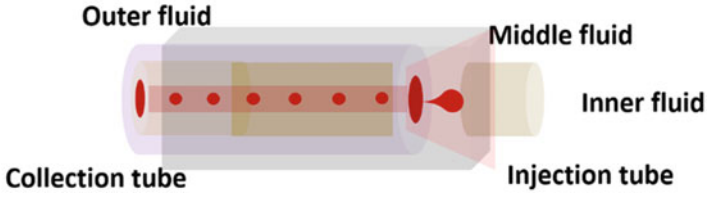

Fig. 5.4 Capillary flow approach in a microfluidic device

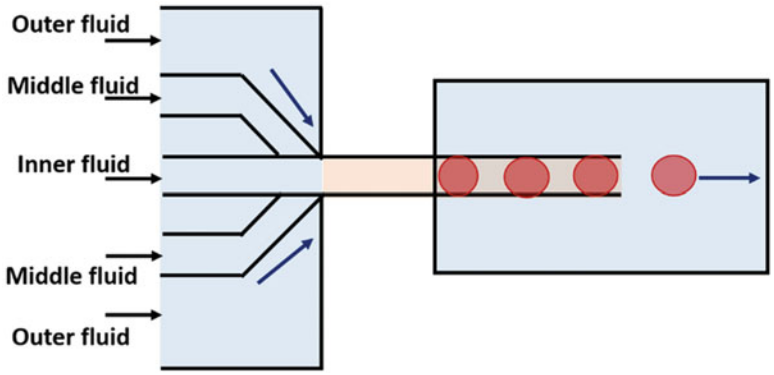

Fig. 5.5 Planar flow approach in a microfluidic device

orifice [42]. This is a one-step process to prepare double emulsions. Commonly, polyurethane is used for making this setup using the soft lithography technique.

3. Water-oil-water double emulsions: For production of these emulsions, Pyrex glass chips have been used [40]. The microfluidic device can have varying flow rates and channel sizes which vary emulsion drop properties. It comprises two T-junction channels, where the first junction is hydrophobic in nature, responsible for the formation of water-in-oil drops and the second junction is hydrophilic in nature, responsible for water-oil-water drops, leading to the formation of a double emulsion. Recent advances in drug delivery systems can certainly profoundly influence the therapeutic outcome, thereby patient compliance.

5.3.1.2.2.3 3D Devices

For the production of emulsions, microfluidic devices of three-dimensional nature can be fabricated by bonding identical molds face-to-face on top of each other. It is made non-planar in nature and has two sequential junctions in each mold. It protects the shell by minimizing contact of droplets to the channel walls and minimizing the channels wetting by the middle phase. It inhibits the disruption of shells in the early polymerization stage [43, 44]. The sequential junctions have different depth channels, a shallow and a deep one. A non-planar junction is formed at their intersection. Water-in-oil or oil-in-water droplets are initially generated in the unmodified planar junction with hydrophobic or hydrophilic walls, respectively, and further passed to a non-planar junction for encapsulation.

5.3.1.2.2.4 Multi-compartment Double Emulsions

Emulsions can be made with distinct inner drops using hydrophobic upstream and hydrophilic downstream T junctions [42]. Two different aqueous solutions are injected in the oil phase at the upstream junction via opposite side channels, whereas at the downstream junction, the same oil phase encapsulates the two cores.

5.3.1.2.3 Tuning of Microchannels

The wettability of the collection channel needs to be tuned for controlled production of emulsions. Tuning is performed by providing a greater affinity of drops toward the continuous phase rather than the dispersed phase. Some of the important methods to improve the wettability of microchannels are the flow confinement method, photoreactive sol–gel coating, and plasma treatment [42].

5.3.2 Protein-Based Nanocarriers

Proteins are known to have unique biological properties and are involved in the major functioning of cell metabolism. Protein-based materials are sensitive and highly complex. They have excellent particle biocompatibility and can be modified with specific ligands for proper drug targeting and binding of drugs [45]. Protein-based nanoparticles are increasingly being used these days to increase stability in microdroplets with lesser adverse effects. Additional benefits include low immunogenic response and can travel through cells via endocytosis [46].

Commonly, bovine serum albumin (BSA), human serum albumin (HSA), and silk protein fibroin are used to form nanocarriers. Fibroin compromises 65–85% of the total protein in silk fibers. The protein has good flexibility, mechanical strength, availability, stability, and biocompatibility. It has low immunogenicity. Human serum albumin is a globular protein made up of 585 amino acids with ligand binding properties. It can be used to load a range of drugs with medium to high concentrations [46]. Gliadin is poorly soluble in water, giving way for the controlled release of hydrophobic and amphiphilic drugs. Another advantage is its ability to attach with mucous membranes allowing for oral and local drug delivery [46].

Co-precipitation, a particle formulation approach based on a protein's differential solubility in two solvents, is often utilized to make protein nanoparticles. Microfluidic systems can be utilized to more precisely control the conditions that govern co-precipitation and generate nanoparticles in a very repeatable manner. Although aqueous and organic phases are still employed to precipitate nanoparticles, microfluidic technologies allow for more precise mixing at the point where the two solvent streams intersect [47]. The solvent's laminar flow regime and regular flow patterns generate consistent mixing conditions, resulting in uniformly sized particles.

5.3.2.1 Methods for Preparing Protein Nanoparticles Using Microfluidic Devices

5.3.2.1.1 Self-Assembly Method (Self-Agglutination of Proteins)

It is a prevalent method involving a protein solution and a desolvating agent. The exposed hydrophobic and reactive residues of proteins are destabilized upon drop-by-drop addition of a desolvating agent, which enhances molecular interactions, creating small aggregates of particles [45]. A commonly used desolvating agent is ethanol.

An extension of this is the microfluidic co-flow method, where instead of adding the desolvating agent dropwise, it flows adjacent to the protein solution. Moreover, additional usage of increased desolvating concentration or use of crosslinkers for stability is not required. In the microfluidic device, protein is introduced in the middle inlet on microfluidic chips, and a desolvating agent surrounds it. A water stream is added as the outer layer to pinch the desolvating agent and protein streams for faster diffusion between them. Mixing is uniform and faster using a microfluidic approach. In addition, the presence of a water layer around protein prevents its contact with PDMS, which inhibits the surface adherence due to the hydrophobic nature of PDMS [30]. A 3D-designed device can allow streams to flow within one another, thus ensuring complete envelopment by water. Size distribution is affected by the concentration of desolvating agents with respect to the protein flow rate ratio. The more the flow rate of the agent, the more concentration in the resulting mixture, and therefore yields the bigger nanoparticle. This method is preferred for in vivo delivery, as crosslinkers are usually slightly toxic and can have adverse health effects [48].

5.3.2.1.2 Using Y-Shaped Microchannels

For example, the use of microfluidic devices with Y-shared microchannel and staggered herringbone micromixers has been used to synthesize Zein protein nanoparticles reproducibly. Zein, a plant-derived natural protein, outperforms other synthetic polymers in regulated drug and therapeutic delivery systems. Pharmaceutical drug targeting, vaccine development, tissue engineering, and gene delivery are only a few of the medical applications [49]. In the first inlet of the microchannel, the filtered Zein solution was loaded as the organic phase into a 1.0 mL syringe, while the aqueous phase was fed into a 3.0 mL syringe in the second inlet channel. The sample was collected at the microfluidics chip's outlet channel at varied total flow rates (TFR) and relative flow rates [50]. When the total flow rate was increased, the nanoparticles produced were substantially smaller, and when it was dropped, the nanoparticles produced were significantly larger. Overall, a higher overall flow rate reduces the size of the droplet created during the solubility phase breakup, and thus the number of nuclei that can be formed within the droplet [47]. The size of the nanoparticle generated shrinks dramatically when the relative flow rate is increased.

5.3.2.2 Recent Applications

The use of peptides in a microfluidic system to make nanomedicines is a revolutionary strategy that has already shown great promise in the field of nanoformulation. The COVID-19 virus accelerated research toward RNA-based vaccinations in 2020 since many firms depended on genetically sequenced peptides for vaccine efficacy. Microfluidics has been found to be an effective method of RNA encapsulation with acceptable synthetic properties when used to synthesize RNA-based vaccines. Microfluidics can provide both a platform for identifying targets via sequence isolation and a synthesis mechanism for preparing the final vaccine. Because of the system's high throughput capacity, several tests can be completed in a short

amount of time [51]. As mentioned above, there has been a tremendous amount of improvement in the research of protein nanoparticles over the years. Nevertheless, more work is still needed on their performance and treatment efficiency to showcase their full potential as a delivery system.

5.3.2.3 Liposomes

A liposome is an amphiphilic lipid vesicle, considered a promising candidate for drug delivery because of its lipid arrangement and resemblance to biological cell membranes. These are generally small and can vary in size, with dimensions usually ranging between nanometers and micrometers. A microfluidic device has made it easier to produce monodisperse liposomes/nanoliposomes and therefore reduces the number of steps and time required in the preparation of liposomes. Droplet-based microfluidic devices are among the popular approaches used in liposome production [52]. It relies on handling two immiscible phases to generate droplets between a micrometer and sub-micrometer size (generally for water-in-oil emulsions), and subsequently, liposomes are formed in droplets [37]. Pressure-controlled flow development devices are manufactured for generating lipid vesicles with a radius less than 50 nm. Controlled pressure systems can yield droplets with both nano size and micron size. They flow in continuous and dispersed phases for acoustic droplet vaporization applications [53].

A double emulsion generated in this system can be transformed into liposomes after solvent extraction and capillary control of non-mixable fluxes. Using a continuous flow system, microfluidic hydrodynamic focusing (MHF) is a simple method to produce 50–500 nm in size liposomes. MHF is based on improving the traditional method of injecting alcohol. It was first designed as a chip consisting of four intersecting microchannels and a solvent containing dispersed lipids. Figure 5.6 shows the preparation of liposomes using microfluidics.

Fabrication of the microfluidic chip has a vital role in the determination of the size of liposomes. The size of the liposome plays a very crucial role in its cellular uptake [3]. The hydrodynamic flow (HFF) method was used to synthesize self-assembled monodisperse liposomes, and the effect of the size of liposomes was studied on the mechanisms of cell absorption. When tested against endocytosis inhibitors, small liposomes followed dynamin-dependent endocytosis, whereas large liposomes followed clathrin-dependent endocytosis. Liposomes have been widely used in cosmetics, pharmaceuticals, food, and agriculture. The use of liposomes has been successful in entrapping a variety of biologically active (unstable) molecules,

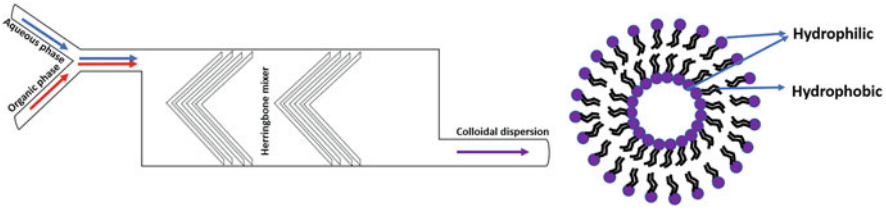

Fig. 5.6 Preparation of liposomes using microfluidics

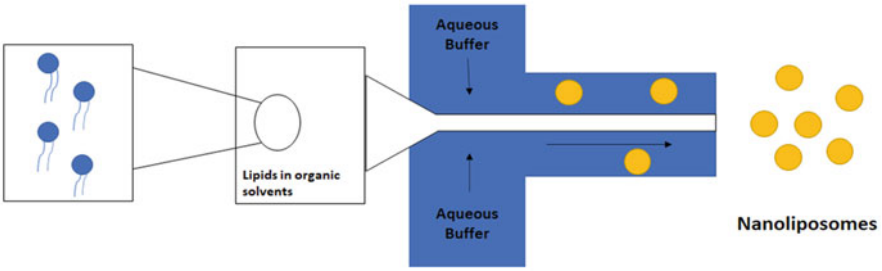

Fig. 5.7 Preparation of liposomes using microfluidics

including tumor-fighting agents, antimicrobials, peptides and proteins, vaccines, and genetic materials [54]. Cationic liposomes are promising carriers for gene vectors due to their ability to form electrostatic complexes with DNA.

Nanoliposomes are similar to liposomal formulations and vary only in terms of size, which can also be prepared using microfluidic devices. The nanoliposomes are lipid vesicles with an average size of 100 nm. They usually consist of a shell and an aqueous core composed of phosphate bilayers. Hydrophobic effects of phospholipids function to induce self-assembled liposomes and minimize interactions between hydrophobic chains and the surrounding aqueous phase, which is entropically unfavorable. There are several applications of nanoliposomes in pharmaceutical fields, as they can encapsulate hydrophilic or hydrophobic drugs, with aqueous cores for hydrophilic drugs and lipid bilayers for hydrophobic drugs [55]. Typically, nanoliposomes are produced using the Y-type microfluidic device. An alcohol solution containing lipids, cholesterol, dioleoyl phosphatidylcholine (DOPC), and dipalmitoyl phosphatidylcholine (DPPC) is injected into a central microfluidic channel. As the lipid solution is injected, it is intersected and sheathed by two streams of aqueous solution injected coaxially. As a result, the lipid solution flows are concentrated hydrodynamically in a thin sheet with either a rectangular or a circular cross-section and results in the diffusion of molecular species from its solvent (alcohol) to non-solvent (water). After an alcohol–water exchange, the alcohol concentration is lowered below the lipid solubility limit, leading to the salting out of lipids from alcohol.

Nanoliposomes continuously self-assemble within microfluidic channels as undissolved lipids self-assemble. By altering the flow ratios of alcohol and water solutions, the size of nanoliposomes can be controlled. By increasing the flow rate, it was demonstrated that the size of nanoliposomes was decreased from 80 to 15 nm. Figure 5.7 shows the preparation of liposomes using a microfluidics device.

Even with the advancement of technology, formulating liposomes at the nano-scale level using microfluidic devices is still challenging. Some of the major drawbacks of preparing liposomes using microfluidics involved (a) Scalability issue in terms of process and equipment, (b) Difficulty in the quantification of residual solvents, (c) Interpretation of correlation between drug encapsulation efficiency and liposomal size [56].

5.3.2.4 Niosomes

Niosomes are made up of bilayers of nonionic surfactant molecules, which are usually a mixture of cholesterol and diacetyl phosphate [57]. Compared to their liposomal counterparts, increased stability, minimal toxicity, and low cost of niosomes make them an attractive alternative for biotechnological and pharmaceutical companies. A typical traditional manufacturing method for niosomes involves mixing two liquid phases in bulk quantities, and therefore they show poor polydispersity and poor reproducibility. A microfluidic device with a T junction is designed and used to produce niosomes. Organic phase such as chloroform was injected into central channels with dissolved surfactants like span 60, cholesterol, and distearoyl phosphatidylethanolamine (DSPE)-PEG 2000 maleimide. An acidic aqueous solution was injected from the bilateral channel to facilitate surfactant self-assembly. All microfluidic channels were heated to 65 °C, and the resultant nanostructures were dialyzed against water for 4 h to generate niosomes. The niosomes were produced with a mean diameter and a narrow size distribution. Furthermore, the size and size distribution can be adjusted by varying the ratio between aqueous and organic flows within the organic phase. Controlled drug administration could be achieved in niosomal preparation using microfluidics. There are many challenges associated with the preparation of niosomes using microfluidic devices. In one such case, the use of high pressure and high volume could lead to the deformation of fabricated PDMS microfluidic devices. It affects the flow rate in the device and subsequently affects the formation of niosomes [58].

5.3.2.5 Micelles

Polymeric micelles are gaining attention due to their simple design, easy formation, and drug-carrying ability [59]. They are formed as self-assembling amphiphilic block copolymers. The polymer consists of a hydrophobic core with a hydrophilic outer shell. Micellar structure enables the encapsulation of poorly water-soluble drugs in hydrophilic segments. Various block copolymers are used to prepare drug-loaded micelles such as Soluplus and poloxamer [60].

The micelles were developed using the continuous flow microfluidic nanoprecipitation method. In brief, the drug and the polymer were solubilized to a specific ratio in the organic phase. The distilled water was taken as an aqueous phase in pump "A" of the microfluidic syringe pump. The drug and the polymer with the organic phase were supplied by pump "B." The pump "A" was divided into two channels by a "T" junction as shown in the diagram, further pumps A and B were connected to the micromixer chip. The flow was monitored through syringe pumps where pump A was regulated at 250 μL/min and pump B at 50 μL/min. In the micromixer chip, the water flow compresses and shrinks the organic phase's flow due to the hydrodynamic flow's focus strategy. It then enters the herringbone mixer into the microchip of the micromixer, and further diffusion of rapid solvent exchange causes the formation of micelles. These micelles were collected and agitated overnight to evaporate the solvent and then dialyzed for 6 h against distilled water to remove the un-entrapped free drug. Subsequent to this, the nanocarriers were freeze-

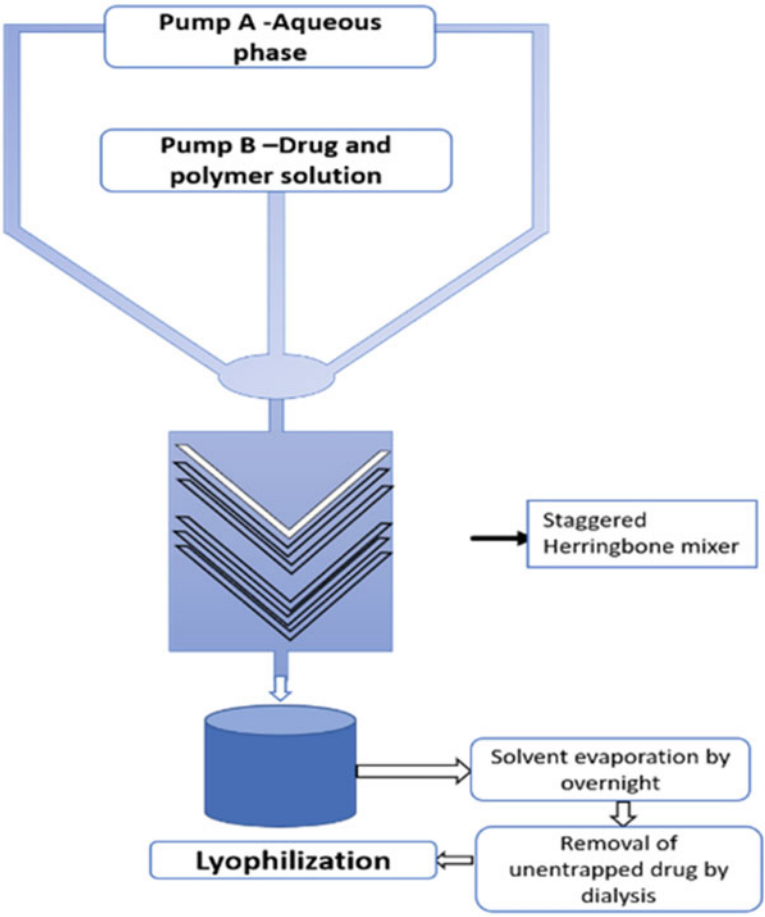

Fig. 5.8 Preparation of micelles using microfluidics

dried and stored at 4 °C. Figure 5.8 shows the preparation of micelles using microfluidics [61].

5.3.3 Polymeric and Hybrid Nanoparticles

Polymeric and hybrid nanoparticles are core–shell nanoparticle structures composed of polymer cores and lipid/lipid-PEG shells that exhibit complementary properties of both polymeric nanoparticles and liposomes, notably in terms of physical stability and biocompatibility. Furthermore, lipid polymeric nanoparticles have recently been shown to have higher in vivo cellular delivery effectiveness when compared to polymeric nanoparticles and liposomes.

5.3.3.1 Polymeric Nanoparticles

Polymeric nanoparticles are a type of colloidal nanocarrier composed of polymers and are used widely in drug administration. Polymeric nanoparticle size is optimized by controlling the mixing time of liquids using microfluidics [38]. For example, chitosan nanoparticles are made using the Y-type microfluidic device. The curcumin potassium hydroxide solution and chitosan acetic acid solution were injected into the microchannels of the type Y device as model drugs and matrix materials, respectively. Nanoparticles were collected at different axial distances to adjust the residence time of the reagents in the microchannels. Nanoparticle properties can be optimized by adjusting the flow rate, concentration, and pH values of drugs and polymers. Figure 5.9 shows the schematic representation of the preparation of microfluidic-based PLGA-lipid-PEG nanoparticles.

Optimized chitosan nanoparticles had a mean diameter of 115 nm, a positive surface load (48 mV), and a high curcumin load of approximately 72%. It significantly improved the solubility and bioavailability of curcumin. In addition, polylactic, glycolic acid-polyethylene glycol (PLGA-PEG) nanoparticles are also manufactured by carrying out nanoprecipitation in microfluidic channels. Acetonitrile is used as the good solvent, while water is used as the poor solvent. Increased ratio of water-to-acetonitrile (10:1 to 10:0.3) and decreased mixing time between the two (0.4 and 0.04 ms) can result in smaller-sized PLGA-PEG nanoparticles. The particle size of developed nanoparticles was reduced from 29 to 23 nm, which can be further reduced to 20 nm, even when the concentration is 20 g/L and the mixing time is 0.04 ms instead of 0.4 ms. Based on these results, the microfluidics technique can significantly enhance the size and size distribution of nanoparticles. Additionally, the pH value and the concentration ratio of the material can be altered to optimize the other properties of the solution [62].

5.3.3.2 Polyionic Complex

Polyionic complexes, for example, polyethylenimine-DNA (pDNA) complexes, are widely used in gene delivery and vaccine delivery. Microfluidics has unique

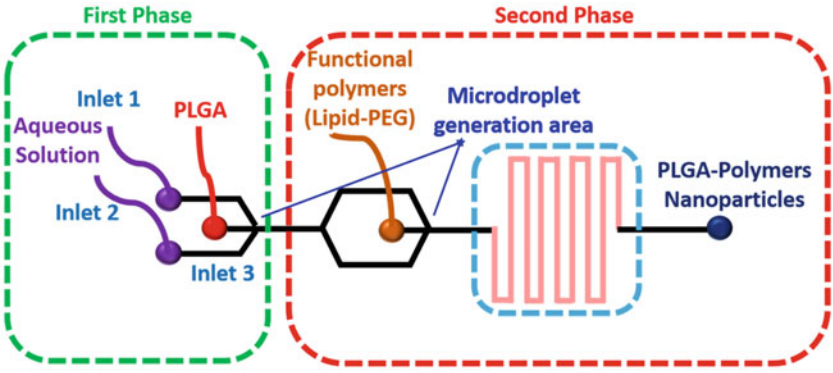

Fig. 5.9 Preparation of microfluidic-based PLGA-Lipid-PEG nanoparticles

capabilities to control the mixture of liquids and assemble poly ionic complexes [63]. Moreover, pDNA/cationic liposome (CL) lipoplexes were also developed using the microfluidic channels. As a result of microfluidic devices, lipoplexes have smaller, more homogeneous structures and a molar charge ratio (R) of 1.5, which implies higher lipid content. In addition, microfluidic lipoplexes were found to have smaller structures with fewer layers (N-2.5) when compared to the pDNA prepared using bulk mixing, which has more layers (N-5).

5.3.3.3 Nanocapsules

A nanocapsule has a strong potential for drug delivery due to its higher surface-to-volume ratio than a microcapsule. Preparation of polymeric nanocapsules may be accomplished by chemically precipitating monomers or polymers at an interfacial interface. Microfluidics is also used to make nanocapsules for drug delivery. It has already been used to fabricate the dendritic polyethylene/pluronic nanocapsules [40]. Dendritic polyethylene, pluronic, and drugs were mixed with water and dissolved in tetrahydrofuran (THF), and injected into microfluidic devices with Y-shaped microchannel separately. THF flows through the water stream, creating a focused solution. Vitamin E and lecithin were dissolved in ethanol (organic phase), whereas chitosan was dissolved in the aqueous phase. In a microfluidic device, the circulation of the two phases regularly produces monodisperse nanocapsules made from chitosan and vitamin E. Additionally, by modifying the flow rates of both phases, it is possible to adjust the size of the fabricated nanocapsules. An increase in flow rate could lead to the formation of smaller-sized nanocapsules. The preparation of nanocapsules by microfluidics is also facilitated by applying external technologies, such as UV lighting, heating, and cooling, to improve the self-assembly of polymers. Figure 5.10 shows the schematic representation of the preparation of nanocapsules.

5.4 Conclusion

Since its inception, microfluidics has progressed with technological advancements, and the microfluidic application field has expanded to include a wide range of disciplines. Biological and medical applications are the major focus of current research, along with other areas. Microfluidics is a multidisciplinary area that necessitates working cohesively between several fields, including engineering, physical sciences, and biological sciences, to continuously improve and find materials whose benefits outweigh the disadvantages and concerns. Microfluidics technology has gained wide attention and demonstrated its use as a drug delivery platform for testing and screening the therapeutic potential of biologically active chemicals and cell–drug carrier interactions. The exceptional control over particle size and composition in microfluidic devices has established it to be a dependable and reproducible resource for producing monodisperse, microencapsulated medicines. Generally, microchannels are produced and calibrated to a specific geometric design, which may not be ideal for other types of formulations. As a

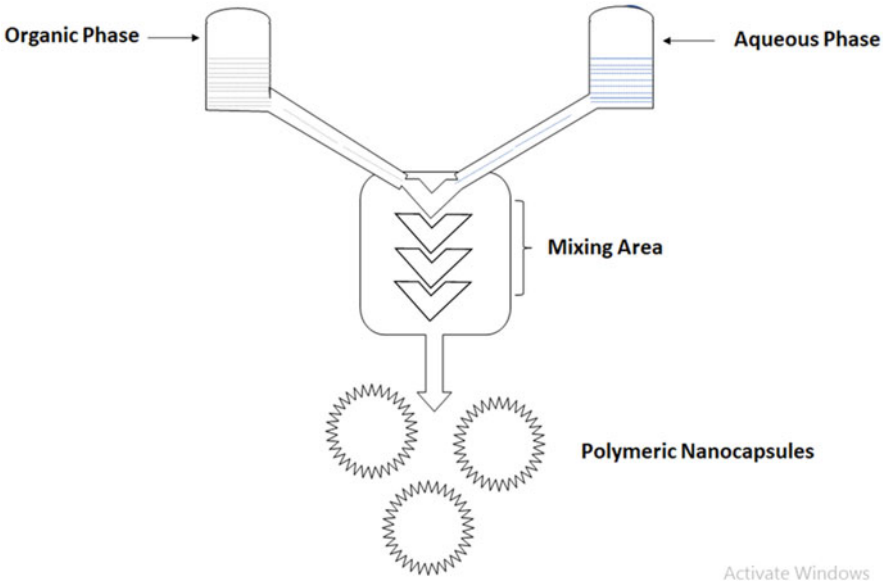

Fig. 5.10 Schematic representation of the preparation of nanocapsules

result, each product is tailored to a particular use, hindering the scalability of microfluidic devices in drug delivery applications. A challenge of microfluidic devices in the geometric aspect is because of the microchannel minuscular dimensions and the high flow rates. Additionally, depositions can occur in the microchannels, causing the mixing conditions to be altered. Therefore, much effort is still needed to improve the application of microfluidics in drug delivery. However, drug delivery will continue to be a major field of study, with various problems and possibilities to create novel technology-based solutions. When combined with its scalability and the rising availability of hardware solutions that support its production, microfluidics can provide an environment for experimentation and exploration in the coming years.

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References

1. Souto EB, Dias-Ferreira J, López-Machado A, Ettcheto M, Cano A, Espuny AC, Espina M, Garcia ML, Sánchez-López E (2019) Advanced formulation approaches for ocular drug delivery: state-of-the-art and recent patents. Pharmaceutics 11(9):1–29. https://doi.org/10.3390/pharmaceutics11090460
2. Kamaly N, Xiao Z, Valencia PM, Radovic-Moreno AF, Farokhzad OC (2012) Targeted polymeric therapeutic nanoparticles: design, development and clinical translation. Chem Soc Rev 41(7):2971–3010. https://doi.org/10.1039/c2cs15344k

3. Andar AU, Hood RR, Vreeland WN, Devoe DL, Swaan PW (2014) Microfluidic preparation of liposomes to determine particle size influence on cellular uptake mechanisms. Pharm Res 31(2): 401–413. https://doi.org/10.1007/s11095-013-1171-8
4. Puneeth SB, Kulkarni MB, Goel S (2021) Microfluidic viscometers for biochemical and biomedical applications: a review. Eng Res Express 3(2). https://doi.org/10.1088/2631-8695/abfd47
5. Kulkarni MB, Goel S (2021) A review on recent advancements in chamber-based microfluidic PCR devices. In: Microelectronics and signal processing. CRC Press, Boca Raton, FL, pp 49–70
6. Zhu H, Korabečná M, Neužil P (2020) Review PCR past, present and future. Biotechniques 69(4):1–10
7. Kulkarni MB, Goel S (2020) Advances in continuous-flow based microfluidic PCR devices—a review. Eng Res Express 2(4):042001. https://doi.org/10.1088/2631-8695/abd287
8. Kulkarni MB, Goel S (2020) Microfluidic devices for synthesizing nanomaterials—a review. Nano Exp 1(1):1–30
9. Baird CL, Myszka DG (2001) Current and emerging commercial optical biosensors. J Mol Recognit 14(5):261–268. https://doi.org/10.1002/jmr.544
10. Afrin S, Gupta V (2021) Pharmaceutical formulation. StatPearls, Treasure Island, FL
11. Kothuru A, Hanumanth Rao C, Puneeth SB, Salve M, Amreen K, Goel S (2020) Laser-induced flexible electronics (LIFE) for resistive, capacitive and electrochemical sensing applications. IEEE Sensors J 20(13):7392–7399. https://doi.org/10.1109/JSEN.2020.2977694
12. Mancera-Andrade EI, Parsaeimehr A, Arevalo-Gallegos A, Ascencio-Favela G, Parra-Saldivar R (2018) Microfluidics technology for drug delivery: a review. Front Biosci 10(1):74–91. https://doi.org/10.2741/e809
13. Wang Y, Kohane DS (2017) External triggering and triggered targeting strategies for drug delivery. Nat Rev Mater 2:17020. https://doi.org/10.1038/natrevmats.2017.20
14. Microfluidics as tool for drug delivery (5). https://www.elveflow.com/microfluidic-reviews/general-microfluidics/microfluidics-as-a-tool-for-drug-delivery/
15. Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, Deng Y (2015) A review on phospholipids and their main applications in drug delivery systems. Asian J Pharm Sci 10(2):81–98. https://doi.org/10.1016/j.ajps.2014.09.004
16. Kulkarni MB, Yashas, Enaganti PK, Amreen K, Goel S (2020) Internet of Things enabled portable thermal management system with microfluidic platform to synthesize MnO_2 nanoparticles for electrochemical sensing. Nanotechnology 31(42):1–8. https://doi.org/10.1088/1361-6528/ab9ed8
17. Kulkarni MB, Goyal S, Dhar A, Sriram D, Goel S (2021) Miniaturized and IoT enabled continuous-flow based microfluidic PCR device for DNA amplification. IEEE Trans Nanobioscience 1241(c):1. https://doi.org/10.1109/tnb.2021.3092292
18. Zhang C, Xing D (2007) Miniaturized PCR chips for nucleic acid amplification and analysis: latest advances and future trends. Nucleic Acids Res 35(13):4223–4237. https://doi.org/10.1093/nar/gkm389
19. Ren K, Zhou J, Wu H (2013) Materials for microfluidic chip fabrication. Acc Chem Res 46(11): 2396–2406. https://doi.org/10.1021/ar300314s
20. Lei KF (2012) Microfluidic systems for diagnostic applications: a review. J Lab Autom 17(5): 330–347. https://doi.org/10.1177/2211068212454853
21. Microfluidics in drug discovery: an overview (2013). https://www.researchgate.net/publication/256706001
22. Damiati S, Kompella UB, Damiati SA, Kodzius R (2018) Microfluidic devices for drug delivery systems and drug screening. Genes 9(2):103. https://doi.org/10.3390/genes9020103
23. Rao LT, Rewatkar P, Dubey SK, Javed A, Goel S (2020) Automated pencil electrode formation platform to realize uniform and reproducible graphite electrodes on paper for microfluidic fuel cells. Sci Rep 10(1):1–9. https://doi.org/10.1038/s41598-020-68579-x

24. Kulkarni MB, Salve M, Goel S (2021) Miniaturized thermal monitoring module with CO laser ablated microfluidic device for electrochemically validated DNA amplification. IEEE Trans Instrum Meas 70(c):1–8. https://doi.org/10.1109/TIM.2021.3097861
25. Kulkarni MB, Goel S (2021) Miniaturized DNA amplification platform with soft-lithographically fabricated continuous-flow PCR microfluidic device on a portable temperature controller. Microfluid Nanofluid 25(8):1–13. https://doi.org/10.1007/s10404-021-02473-4
26. Srikanth S, Dudala S, Jayapiriya US, Mohan JM, Raut S, Dubey SK, Ishii I, Javed A, Goel S (2021) Droplet-based lab-on-chip platform integrated with laser ablated graphene heaters to synthesize gold nanoparticles for electrochemical sensing and fuel cell applications. Sci Rep 11(1):1–12. https://doi.org/10.1038/s41598-021-88068-z
27. Teh S-Y, Lin R, Hung L-H, Lee AP (2008) Droplet microfluidics. Lab Chip 8(2):198–220. https://doi.org/10.1039/B715524G
28. Hong M, Zhu S, Jiang Y, Tang G, Pei Y (2009) Efficient tumor targeting of hydroxycamptothecin loaded PEGylated niosomes modified with transferrin. J Control Release 133(2):96–102. https://doi.org/10.1016/j.jconrel.2008.09.005
29. Gorantla S, Rapalli VK, Waghule T, Singh PP, Dubey SK, Saha RN, Singhvi G (2020) Nanocarriers for ocular drug delivery: current status and translational opportunity. RSC Adv 10(46):27835–27855. https://doi.org/10.1039/d0ra04971a
30. Pla-Roca M, Fernandez JG, Mills CA, Martínez E, Samitier J (2007) Micro/nanopatterning of proteins via contact printing using high aspect ratio PMMA stamps and nanoimprint apparatus. Langmuir 23(16):8614–8618. https://doi.org/10.1021/LA700572R
31. Park J, Li J, Han A (2010) Micro-macro hybrid soft-lithography master (MMHSM) fabrication for lab-on-a-chip applications. Biomed Microdevices 12(2):345–351. https://doi.org/10.1007/S10544-009-9390-9
32. Agrawal A, Kulkarni S, Sharma SB (2016) Recent advancements and applications of multiple emulsions. Adv Pharm. https://doi.org/10.7439/ijap
33. Tomeh MA, Zhao X (2020) Recent advances in microfluidics for the preparation of drug and gene delivery systems. Mol Pharm 17(12):4421–4434. https://doi.org/10.1021/acs.molpharmaceut.0c00913
34. Shah RK, Shum HC, Rowat AC, Lee D, Agresti JJ, Utada AS, Chu LY, Kim JW, Fernandez-Nieves A, Martinez CJ, Weitz DA (2008) Designer emulsions using microfluidics. Mater Today 11(4):18–27. https://doi.org/10.1016/S1369-7021(08)70053-1
35. Costa L, Reis RL, Silva-Correia J, Oliveira JM (2020) Microfluidics for angiogenesis research. Adv Exp Med Biol 1230:97–119
36. Javaid MU, Cheema TA, Park CW (2017) Analysis of passive mixing in a serpentine microchannel with sinusoidal side walls. Micromachines 9(1):8. https://doi.org/10.3390/mi9010008
37. Carugo D, Bottaro E, Owen J, Stride E, Nastruzzi C (2016) Liposome production by microfluidics: potential and limiting factors. Sci Rep 6(1):1–15. https://doi.org/10.1038/srep25876
38. Brannigan RP, Dove AP (2016) Synthesis, properties and biomedical applications of hydrolytically degradable materials based on aliphatic polyesters and polycarbonates. Biomater Sci 5(1):9–21. https://doi.org/10.1039/C6BM00584E
39. Shepherd SJ, Issadore D, Mitchell MJ (2021) Microfluidic formulation of nanoparticles for biomedical applications. Biomaterials 274:120826. https://doi.org/10.1016/J.BIOMATERIALS.2021.120826
40. Hasani-Sadrabadi MM, Karimkhani V, Majedi FS, Van Dersarl JJ, Dashtimoghadam E, Afshar-Taromi F, Mirzadeh H, Bertsch A, Jacob KI, Renaud P, Stadler FJ, Kim I (2014) Microfluidic-assisted self-assembly of complex dendritic polyethylene drug delivery nanocapsules. Adv Mater 26(19):3118–3123. https://doi.org/10.1002/ADMA.201305753
41. Palanisamy P, Raichur AM (2009) Synthesis of spherical NiO nanoparticles through a novel biosurfactant mediated emulsion technique. Mater Sci Eng C 29(1):199–204. https://doi.org/10.1016/j.msec.2008.06.008

42. Vladisavljević GT, Al Nuumani R, Nabavi SA (2017) Microfluidic production of multiple emulsions. Micromachines 8(3). https://doi.org/10.3390/mi8030075
43. Puneeth SB, Goel S (2019) Novel 3D printed microfluidic paper-based analytical device with integrated screen-printed electrodes for automated viscosity measurements. IEEE Trans Electron Devices 66(7):3196–3201. https://doi.org/10.1109/TED.2019.2913851
44. Puneeth SB, Goel S (2019) Chemical and biological sensors amperometric automation and optimization paper microfluidic viscometer. IEEE Sens Lett 3(3):1–4. https://doi.org/10.1109/LSENS.2019.2894623
45. Hakala TA, Davies S, Toprakcioglu Z, Bernardim B, Bernardes GJL, Knowles TPJ (2020) A microfluidic co-flow route for human serum albumin-drug–nanoparticle assembly. Chemistry 26(27):5965–5969. https://doi.org/10.1002/chem.202001146
46. Hong S, Choi DW, Kim HN, Park CG, Lee W, Park HH (2020) Protein-based nanoparticles as drug delivery systems. Pharmaceutics 12(7):1–28. https://doi.org/10.3390/pharmaceutics12070604
47. van Ballegooie C, Man A, Andreu I, Gates BD, Yapp D (2019) Using a microfluidics system to reproducibly synthesize protein nanoparticles: factors contributing to size, homogeneity, and stability. Process 7(5):290. https://doi.org/10.3390/PR7050290
48. Evanko DS, Haydon PG (2005) Elimination of environmental sensitivity in a cameleon FRET-based calcium sensor via replacement of the acceptor with Venus. Cell Calcium 37(4):341–348. https://doi.org/10.1016/j.ceca.2004.04.008
49. Labib G (2018) Overview on zein protein: a promising pharmaceutical excipient in drug delivery systems and tissue engineering. Expert Opin Drug Deliv 15(1):65–75. https://doi.org/10.1080/17425247.2017.1349752
50. Alqahtani AY, Rajkhan AA (2020) E-learning critical success factors during the covid-19 pandemic: a comprehensive analysis of e-learning managerial perspectives. Educ Sci 10(9):1–16. https://doi.org/10.3390/educsci10090216
51. Weaver E, Uddin S, Cole DK, Hooker A, Lamprou DA (2021) The present and future role of microfluidics for protein and peptide-based therapeutics and diagnostics. Appl Sci 11(9):4109. https://doi.org/10.3390/app11094109
52. Ma Q, Cao J, Gao Y, Han S, Liang Y, Zhang T, Wang X, Sun Y (2020) Microfluidic-mediated nano-drug delivery systems: from fundamentals to fabrication for advanced therapeutic applications. Nanoscale 12(29):15512–15527. https://doi.org/10.1039/d0nr02397c
53. Davies RT, Kim D, Park J (2012) Formation of liposomes using a 3D flow focusing microfluidic device with spatially patterned wettability by corona discharge. J Micromech Microeng 22(5):055003. https://doi.org/10.1088/0960-1317/22/5/055003
54. Fan Y, Zhang Q (2013) Development of liposomal formulations: from concept to clinical investigations. Asian J Pharm Sci 8(2):81–87. https://doi.org/10.1016/j.ajps.2013.07.010
55. Liu D, Zhang H, Fontana F, Hirvonen JT, Santos HA (2018) Current developments and applications of microfluidic technology toward clinical translation of nanomedicines. Adv Drug Deliv Rev 128:54–83. https://doi.org/10.1016/J.ADDR.2017.08.003
56. Carugo D, Bottaro E, Owen J, Stride E, Nastruzzi C (2016) Liposome production by microfluidics: potential and limiting factors. Sci Rep 6:25876
57. Ag Seleci D, Maurer V, Stahl F, Scheper T, Garnweitner G (2019) Rapid microfluidic preparation of niosomes for targeted drug delivery. Int J Mol Sci 20(19):4696. https://doi.org/10.3390/IJMS20194696
58. Lo CT, Jahn A, Locascio LE, Vreeland WN (2010) Controlled self-assembly of monodisperse niosomes by microfluidic hydrodynamic focusing. Langmuir 26(11):8559–8566. https://doi.org/10.1021/la904616s
59. Ahmad G, El Sadda R, Botchkina G, Ojima I, Egan J, Amiji M (2017) Nanoemulsion formulation of a novel taxoid DHA-SBT-1214 inhibits prostate cancer stem cell-induced tumor growth, vol 406. Elsevier B.V., Amsterdam

60. Kulkarni MB, Velmurugan K, Prasanth E, Amreen K, Nirmal J, Goel S (2021) Smartphone enabled miniaturized temperature controller platform to synthesize nio/cuo nanoparticles for electrochemical sensing and nanomicelles for ocular drug delivery applications. Biomed Microdevices 23(2):1–13. https://doi.org/10.1007/s10544-021-00567-y
61. Maravajjala KS, Swetha KL, Sharma S, Padhye T, Roy A (2020) Development of a size-tunable paclitaxel micelle using a microfluidic-based system and evaluation of its in-vitro efficacy and intracellular delivery. J Drug Deliv Sci Technol 60:102041. https://doi.org/10.1016/j.jddst.2020.102041
62. Lim JM, Bertrand N, Valencia PM, Rhee M, Langer R, Jon S, Farokhzad OC, Karnik R (2014) Parallel microfluidic synthesis of size-tunable polymeric nanoparticles using 3D flow focusing towards in vivo study. Nanomedicine 10(2):401–409. https://doi.org/10.1016/J.NANO.2013.08.003
63. Belliveau NM, Huft J, Lin PJ, Chen S, Leung AK, Leaver TJ, Wild AW, Lee JB, Taylor RJ, Tam YK, Hansen CL, Cullis PR (2012) Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA. Mol Ther Nucleic Acids 1(8):e37. https://doi.org/10.1038/MTNA.2012.28

Microfluidics in Drug Delivery

6

Rohan Chakraborty and Suhel Parvez

Abstract

Drug delivery through conventional modes of administration (such as oral, intranasal, subcutaneous, transdermal, rectal) is associated with several disadvantages which often nullify the therapeutic potential and render the drug ineffective. To circumvent some of these obstacles, microfluidics has emerged as a viable alternative over the past few decades, providing much-needed breakthroughs in the domain of nanotechnology to help formulate nanoparticles for localized drug delivery. This has aided drug development in even the most challenging therapeutic use cases, including regenerative medicine and anticancer treatment, while also holding great potential in multifarious disease models, such as tumors, diabetes, asthma, thalassemia, and atherosclerosis. Microfluidic devices also have significant clinical relevance owing to their structural uniformity, reproducibility, and highly controllable nature—properties which have facilitated novel drug delivery systems with greater stability, efficiency, and sustained-release patterns. Thus, microfluidics can potentially be a game-changer in the domain of drug discovery and holds immense promise with regard to drug formulation, multi-dosing regimens, drug immobilization, and drug delivery systems such as sustained-release formulations and organ-on-a-chip devices. This chapter throws light on microfluidics, their advantages over conventional drug delivery systems, their properties and applications, as well as recent advances and future perspectives.

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Keywords

Drug delivery systems · Microfluidics · Nanomedicine · Microfabrication · Controlled drug delivery · Drug screening

Abbreviations

ADME	Absorption, distribution, metabolism, elimination
API	Active pharmacological ingredient
CED	Convection enhanced delivery
COC	Cyclic olefin copolymer
DDS	Drug delivery systems
FDA	US Food and Drug Administration
FRR	Flow rate ratio
GMP	Good manufacturing practices
HDL	High density lipoprotein
HFF	Hydrodynamic flow focusing
LNPs	Lipid nanoparticles
PDI	Polydispersity index
PDMS	Polydimethylsiloxane
PLGA	Poly(lactic-co-glycolic acid)
PMMA	Polymethyl methacrylate
PTFE	Polytetrafluoroethylene
SC	Stratum corneum

6.1 Introduction

While drug development has always involved cutting-edge research in the quest to enhance the efficacy, bioavailability, and absorption of the drug by the body, coupled with reducing notable adverse effects for establishing a better patient safety profile, progress in the said domain has plateaued over the past few decades [1]. The downsides of conventional modes of drug delivery include poor patient compliance, lackluster absorption and solubility, drug–drug and drug–food interactions, and potential toxic effects [2]. Bulk manufacturing of traditional drug delivery systems (DDS) has several limitations, viz. the requirement of large quantities of raw materials for synthesis of the active pharmacological ingredient (API), finding the right vehicle for optimizing therapeutic potential of the pharmacological agent, and optimizing localized delivery of the API [3–6]. The inability to achieve optimal quality control, especially with regard to interbatch variability in product quality, is also another hurdle associated with traditional DDS, particularly in large-scale manufacturing [7].

To circumvent some of these obstacles, microfluidics has emerged as a viable alternative over the past few decades, providing much needed breakthroughs in the domain of nanotechnology to help formulate nanoparticles for localized drug delivery [8, 9]. This has aided drug development in even the most challenging therapeutic use cases, including regenerative medicine [8] and anticancer treatment, while also holding great potential in multifarious disease models, such as tumors [10], diabetes [11], asthma [12], thalassemia [13], and atherosclerosis [14]. Nanoformulations, which can be tweaked in terms of size and other physicochemical properties, impart several beneficial effects in drug delivery including the ability to release controlled amounts of the drug at staggered time-points, reach their specific target sight efficiently, reduce potential toxic effects [15], and improve the uptake and absorption of the drug at the cellular level [15, 16]. They can also increase the stability of drugs inside the body by preventing premature degradation when used as an encapsulating agent in certain drugs such as antiangiogenic drugs [17]. Another impetus provided by microfluidics is the ability to control highly scalable and reproducible fabrication mechanisms for synthesizing nanoformulations on a large scale.

Thus, microfluidics can potentially be a game-changer in the domain of drug discovery and holds immense promise with regard to drug formulation, multidosing regimens, drug immobilization, and DDS such as sustained release formulations and organ-on-a-chip devices. Their ability to be fine-tuned for targeted release at precise locations with minimal adverse effects stands out in an ocean of various other conventional DDS, and provides significant advantages over existing counterparts. This chapter will throw light on microfluidics, their advantages over conventional DDS, their properties and applications, as well as recent advances and future perspectives.

6.2 Conventional Drug Delivery Systems and Their Drawbacks

Drug delivery through conventional modes of administration, such as oral, intranasal, subcutaneous, transdermal, and rectal, is associated with several disadvantages that often nullify the therapeutic potential and render the drug ineffective. Some of these shortcomings include:

1. **Loss of therapeutic potential before reaching target site**
   Drugs delivered by conventional DDS are typically administered at a site more distant than the target site. As a result, there is often a significant loss of the actual pharmacological product that in turn alters the effectiveness of the drug.
2. **Systemic toxicity at off-target tissues**
   To make matters worse, the drug can potentially impart toxic side effects to other tissues of the body while en route to the target site [18]. The DDS should be tweaked so as to ensure that it is capable of retaining optimal efficiency when it eventually reaches the target site while also minimizing toxicity at off-target sites [19].

3. **Poor therapeutic window**
   Conventional modes of administration (like oral) are typically associated with an initial concentration that is optimal for the requisite efficiency, but later tapers off due to mechanisms such as first-pass metabolism, leading to lower levels of the drug in the bloodstream [20]. If the concentration of the drug in the bloodstream is below the therapeutic threshold, it will be ineffective. On the other hand, it will be toxic if the drug concentration soars over the threshold. Sustained release formulations can help negate this by maintaining a steady state of drug concentration throughout the therapeutic window [21].
4. **Ill-suited for long-term delivery**
   Several treatment regimens necessitate a sustained release of the pharmacological component at fixed amounts over extended periods of time, such as in diabetic patients (who require treatment with insulin), as well as other ailments that require hormonal therapy to mimic the individual's natural pulsatile pattern [21]. Sustained release of DDS requires strategies to fine-tune factors such as the mechanism by which the drug is released, ability to target specific sites, response to physiological stimuli like pH and temperature [22–24].
5. **Poor stability and viability**
   Many drugs administered by conventional DDS are susceptible to enzymatic degradation inside the body (such as the breakdown of protein derivatives by proteolytic enzymes). Some are also affected by the body's immune response, and thus, it limits the effectiveness of such pharmacological compounds [25]. Several strategies have been used to bypass these, such as the use of gelatin-coated capsules that are not as easily degraded, thus allowing for sustained release phenomenon [26, 27].
6. **Poor patient compliance**
   Many oral drugs that are subjected to first-pass metabolism and other physiological barriers have a poor therapeutic window that necessitates dosing regimens with multiple intakes—often daily. This not only results in decreased patient compliance, but poses a serious concern for morbid patients with chronic illnesses who require a fixed therapeutic concentration in the body at all times, thus necessitating repeated pharmacological interventions [19, 28].
7. **Short half-lives**
   The half-life of a pharmacological compound can be defined as the time required for the concentration of the drug in the body to reach 50% of its original concentration [29]. The drugs that possess short half-lives typically provide rapid relief for acute conditions but are ill-suited for chronic illnesses. Pharmacological stability in the body is affected by a multitude of factors such as age, basal metabolic rate, and sex of the patient, along with other variables like drug formulation and handling [30]. Other causes of rapid degradation leading to shorter half-lives are physiological and enzymatic mechanisms such as hydrolysis, oxidation, aggregation, denaturation, isomerization, and deamidation if the drug is protein or peptide-based [31].

8. **Inefficient drug targeting**
   The purpose of targeted pharmacological intervention is to ensure significant accrual of the drug at the target site so as to enhance the efficacy and minimize therapeutic loss as well as off-target toxicity [32]. For a targeted DDS to be more efficient, it must be equipped with the properties of targeting, evasion, retention, and elimination [33]. Targeted DDS can be equipped with either active targeting—in which there are drug–tissue or drug–cell interactions by virtue of certain specific ligands/receptors at the target site, or passive targeting—which involves distribution of the drug to different target organs through the bloodstream and extravasation [34, 35]. However, there is some amount of drug buildup in neighboring organs in the case of passive targeting. Active targeting, on the other hand, requires the drug to be in close proximity with the ligand (distances of the order of less than 0.5 nm)—which is dependent on extravasation and the blood circulation itself, thus being indirectly dependent on passive targeting [34]. Thus, for optimal efficacy, such DDS require an extended circulation time within the body so as to enhance the retention of the drug and the interaction at the target side. This can be achieved by structural modification of the pharmacological moiety through the addition of compounds such as polyethylene glycol [36].

In light of these shortcomings of conventional DDS, microfluidic technologies have emerged as a boon for the pharmacological industry owing to their beneficial effects that will be covered in the subsequent sections.

6.3 What Are Microfluidics

Microfluidic technology, which has been steadily growing in stature over the past few decades, utilizes learning from various scientific domains, such as nanotechnology, pharmacology, physics, and biotechnology to engineer small devices (also termed as microdevices) consisting of microchannels and microchambers with sizes of the order of micrometers, which can be fabricated in a manner so as to wield control over the properties and volume of the pharmacological components in them [37, 38].

The advent of microfluidics heralded several advantages over existing systems, especially in reduction of raw materials and reagents, lowering waste production, improving reaction times to the order of seconds, facilitating accurate 3D modeling to mimic the physiological environment of the body for tissue and cell-culture-based analyses, allowing multiplexed as well as high throughput drug screening, enabling the fusion of multiple mechanisms, viz. cell culture, lysis, mixing, and detection on one device [39, 40]. Microfluidics facilitates the fabrication of DDS with microcarriers (with dimensions ranging from a few hundred nm to even a few mm) that can be equipped with precontrolled patterns of release and precise target sites within the body. This enables sustained release of the drug and longer retention times in the body, reduces potential toxic effects, and enhances patient compliance—resulting in a better safety profile [41–43].

The biggest advantage of microfluidic technologies is their ability to engineer microdevices whose properties enable the programmed release of precise payloads [44]. By facilitating the fabrication of nanoparticles containing multiple carriers of several sizes, microfluidics can help achieve simultaneous delivery of more than one drug [41]. Through preprogrammed release patterns based on specific physiological stimuli such as pH and temperature, co-delivery of multiple drugs through the same DDS can be achieved, thereby eliminating the need for repeated drug intakes and reducing patient discomfort as well as potential adverse effects [45]. Apart from these, microfluidics also provides several benefits in both manufacturing and R&D. Increased production efficiency, reduced interbatch variability in product quality, and efficient scaling up of manufacturing resulting in cheaper manufacturing costs of nanoparticles are just some of the various advantages provided by microfluidics in the fabrication of newer DDS [46]. While conventional methods involve techniques such as the mechanical breakdown of bulk constituents and nanoprecipitation, they usually have several shortcomings such as the lack of uniformity in size and greater interbatch variability [47]. On the other hand, microfluidic technologies are usually fabricated by bottom-up and top-down paradigms, which enable greater control over experimental variables, thus negating the aforementioned drawbacks [48].

Microfluidic technologies have also emerged as a potential candidate to partially reduce the requirement of animals in research on account of providing newer in situ platforms such as organ-on-a-chip and cell-on-a-chip to replicate physiological conditions more accurately. Fabrication of microfluidic devices, however, requires the careful consideration of various factors such as the properties of the synthesizers being utilized, the materials used in their fabrication, their compatibility with different carriers, and how many inlets the microfluidic device contains [49]. Two of the most frequently used polymers in the fabrication of microfluidic devices using the soft-lithography method are polydimethylsiloxane (PDMS) and polymethyl methacrylate (PMMA) [50]. PDMS and PMMA can be tweaked in order to facilitate the entry of oxygen, thus rendering them superior to other polymers. However, the downside to using PMMA and PDMS is that they are vulnerable to swelling on exposure to certain solvents (such as acetone), which subsequently alters the flow of fluids within the microfluidic device and can adversely affect the carrier [51]. Some other polymers also in use that can better withstand exposure to such solvents include alternative polymers that are chemically resistant to strong solvents. Cyclic olefin copolymer (COC) and polytetrafluoroethylene (PTFE) are two such polymers. PTFE and COC are typically used to fabricate microfluidics by the hot-embossing method [52]. More than 50 nanoparticle DDS have been formally approved by the US Food and Drug Administration (FDA) in the past few decades [8].

6.4 Properties of Microfluidic Devices Which Affect Drug Delivery Systems

The efficiency of DDS is directly associated with certain physical as well as chemical properties of the microfluidic device, such as size, shape, and structure [10, 53].

6.4.1 Size

Size of the microfluidic device is a key factor in deciding the efficiency and effectiveness of a DDS. The capacity to fabricate microfluidics with the desired uniformity and reproducibility plays a role in attaining DDS of the requisite dimensions. Vital parameters such as how long the drug remains in the bloodstream, its ability to penetrate certain physiological barriers, and distribution of the drug to different organs are all dependent on the size of the microfluidic device [41, 54, 55]. Nanoparticles with diameters <400 nm have been reported to exhibit greater permeability and retention effect. This in turn enables the microfluidic device to passively target certain tumors [56]. While nanoparticles produced by conventional methods through bulk mixing have an average size of 200 nm and polydispersity index (PDI) of 0.4, those fabricated by the superior mixing in microfluidic devices display greater homogeneity in terms of size, with an average particle size of 50 nm and PDI of 0.1 [57]. Likewise, lipid nanoparticles fabricated conventionally in bulk have a diameter of 70 nm, while those engineered by microfluidic technologies are 20 nm in comparison [58].

6.4.2 Shape and Structure

Shape and structure are integral factors not just in the fabrication of a nanoparticle but also in determining their absorption and uptake by the body [59]. Crucial parameters such as the rate at which the drug is released in the bloodstream, their stability and retention in the body, and degradation by physiological triggers like pH can be altered by merely tweaking structural details such as porosity. Studies have demonstrated that nanoparticles with the aforementioned desirable properties can be fabricated using microfluidic technologies by integrating the drug of choice into a polymeric encapsulation composed of acetylated dextran functionalized with folic acid (ADS-FA) [60]. Others have shown that altering the flow rate ratio (FRR) and hydrophobic nature of the chitosan chains can facilitate a change in how compact the chitosan nanoparticle is [61]. DDS that can be preprogrammed to have specific properties are often fabricated using microfluidic devices with certain structural patterns, such as spiral channels, herringbone, and tesla structure [62].

6.4.3 Surface Modification

Microfluidics is more adept at targeted drug delivery than conventional DDS owing to the fact that they can be engineered to contain surface modifications that facilitate targeting specific cells, tissues, and tumors. Studies on cancerous cells have shown that fabrication of hybrid nanoparticles through microfluidic mixing with certain surface modifications—such as encapsulation with certain cationic lipids—helps attain nanoparticles with a consequent size (more than 150 nm) and ξ charge

(+15 mV) that is appropriate for pDNA to successfully reach HeLa cells as well as HEK-293 cells [63].

6.4.4 Elasticity

Elasticity of DDS has been reported to play a crucial role in the release of the pharmacological component, its stability in the body, as well as absorption and uptake by cells and tissues [16]. Microfluidic devices impart the ability to control and tweak parameters such as flow volume of the drug, which can in turn alter the elasticity of the DDS.

6.5 Fabrication of Nanoparticles Using Microfluidics

Microfluidic techniques have paved the way for the fabrication of nanoparticles with multiple pharmacological components having varied properties and release patterns. Microfluidics has helped revolutionize DDS by facilitating the manipulation of the API within these nanodevices, and this section will highlight how such nanocarriers have been engineered using microfluidics.

1. **Double and multiple Emulations**
   When two immiscible liquid phases are mixed together, the resulting formulation is termed as an emulation. Emulations typically consist of a continuous phase and a dispersed phase [64]. Microfluidic techniques can be used to fabricate DDS containing droplets inside other droplets, resulting in the formation of double or multiple emulsions [65]. This facilitates formulation of uniform fluidic droplets with a great capacity for control and manipulation. Emulations have been widely used in both the food as well as the pharmaceutical industry owing to the fact that they can be programmed to influence a plethora of different constituent components [66]. By tweaking properties such as the shape, size, and structure of the emulation, coupled with using differentially responsive components with variable response to physiological stimuli, one can alter the release patterns of the drug inside the body [44]. Fabrication of such emulsions through droplet microfluidic technologies can also help circumvent issues like greater dispersity, inefficient encapsulation, and poor structural uniformity that plague conventional emulsification methods [44]. Studies have shown that microfluidic devices enable the fabrication of double and multiple emulsions with greater stability and retention times by manipulating physicochemical properties such as morphologies of the constituent droplets and their coefficient of variation [67, 68].
2. **Protein or biological membrane-based nanoparticles**
   Owing to concerns over the poor biodegradability and toxic effects of several synthetic polymers, there has been a surge in the use of natural materials like proteins and biological membranes in the fabrication of many DDS

[17, 69]. Naturally available materials such as gelatin, silk fibroin, and collagen have been used in the engineering of many nanoparticles, despite their increased sensitivity and complexity [70, 71].

Studies have shown that protein-based nanoparticles can be programmed so as to have greater retention times in the bloodstream and enhanced drug targeting capabilities, particularly with respect to tumors [72]. The rapid rise in the popularity of microfluidic technologies has helped bridge the gap between small scale laboratory-based production and large-scale industrial manufacturing, especially with regard to the fabrication of biomembrane-based nanoparticles. Several applications of protein-based nanoparticles have been studied—such as the combination of lipid (phospholipid) and protein molecules (apolipoprotein) by microfluidic technology to form nanoparticles that mimic high density lipoprotein (HDL) [73]. This HDL mimicking nanoparticle could be fabricated in such a manner so as to enable its cellular uptake by competitively inhibiting the body's native HDL. The HMG-CoA inhibitor Simvastatin has been encapsulated with components such as fluorescent-labeled agents, gold, and iron oxide to yield the aforementioned faux-HDL, thereby demonstrating the capabilities of microfluidic technologies in not just fabricating efficient targeted DDS but also contain components that aid in fluorescent imaging for validating the precise targeting capabilities of the nanoparticle. Studies have also shown that microfluidic devices help facilitate the fabrication of nanoparticles <100 nm in size with a polydispersity index <0.2, termed as leukosomes, which can mimic leukocytes [74].

3. **Lipid-based nanoparticles**

It has long been known that lipid nanoparticles (LNPs) have a wide ranging spectrum of uses in both DDS as well as the food industry owing to their capacity to load large volumes of components—be it API or food components—within the lipid encapsulation [75, 76]. LNPs hold several beneficial advantages, viz. improved efficiency in drug loading, greater capacity for structural modifications, enhanced biological compatibility [77, 78]. However, for enhanced efficacy in their potential use as DDSs, LNPs can be tweaked to contain surface modifications that increase the stability, circulation time in the bloodstream, and targeted drug delivery at specific target sites [36, 79]. Although conventional methods used in the manufacture of LNPs involve the bulk mixing of different liquid phases or hydration of the lipid layer, there is greater polydispersity and a lack of homogeneity in the generated nanoparticles. Precise fluid dynamics in microfluidic devices helps overcome some of these limitations and enables the fabrication of LNPs with homogeneous sizes and reduced polydispersity [76, 80]. Studies have shown that microfluidic devices engineered with a mix of lipid layer and cationic polymers can be used to fabricate LNPs that encapsulate pDNA. Doing so leads to the generation of homogeneous LNPs and reduces the production time [81]. Others have fabricated LNPs for targeted release at lymph nodes by making the nanoparticles pH sensitive using the herringbone structure inside microfluidic devices [82].

4. **Polymer-based nanoparticles**
   Conventional manufacturing techniques such as evaporation of the solvent, crosslinking of emulsions, mixing of solvents with antisolvents, and ionic gelation can be used to fabricate polymeric nanoparticles [83]. However, fabrication of the same using microfluidic technologies such as millisecond mixing, rapid solvent exchange, and tunable operating conditions yields nanoparticles with greater structural uniformity that are also easier to manipulate for targeted DDS. Several drugs, viz. dexamethasone, amoxicillin, and docetaxel, are delivered via polymeric nanoparticles that are fabricated by microfluidic devices using techniques such as hydrodynamic flow focusing (HFF) [83–85]. Other drugs such as iopamidol can be loaded into polymer-based nanocarriers through microfluidic techniques like droplet-based methods. By maneuvering the shape of the nanoparticles via ionic crosslinking, one can alter the release pattern of the drug in the body [86]. Techniques like HFF can be adopted to fabricate nanoparticles containing a complex constituted of a polymer and pDNA for targeted gene delivery. Studies have shown that such complexes have been successfully used in gene delivery in in vivo studies conducted on mice, with greater efficiency in gene transfection and reduced toxic effects vis-à-vis conventional gene delivery methods [87].
5. **Lipid-polymer hybrid nanoparticles**
   Hybrid nanoparticles are made up of two or more constituent building blocks, typically a lipid and a polymer, and can potentially be programmed to have increased retention in the bloodstream and greater efficiency [81, 88]. Studies have shown that the retention time of these hybrid nanoparticles, which can be fabricated by microfluidic devices using a tesla mixer, can be extended up to 24 h by altering the Poly(lactic-co-glycolic acid) (PLGA) ratio [89, 90]. An added benefit of using such hybrid nanoparticles is that both the lipid covering as well as the inner polymeric material can encapsulate the API. Such simultaneous encapsulation allows the controlled release of multiple pharmacological compounds at different time points [13]. One such example is that of a nanoparticle loaded with two different drugs—an anticancer drug (doxorubicin) and an antiangiogenic drug (sorafenib), which have different release patterns but can be co-encapsulated in the inner polymeric core and the outer lipid covering respectively for differential release in the body [91].
6. **Pharmaceutical nanoparticles**
   Mixing of fluids inside microfluidic channels has facilitated the fabrication of several pharmacological spheres, often colloids, without the need for stabilizers. This has helped produce several nanodrugs, such as the corticosteroid budesonide (typically administered via a nebulizer in the treatment of asthma), which can be engineered using a microfluidic mixer [92]. Microfluidic devices also facilitate the mixing of the pharmaceutical compound in these microchannels, thereby giving rise to nanoformulations with different structural organization and solubility than those manufactured by conventional methods [93].

Table 6.1 Some nanoparticle-encapsulated drugs that are fabricated by microfluidic devices

Drug	Nanomaterial used	Microfluidic device used	Application	References
Doxorubicin	Human serum albumin	T mixer	Liver cancer	[100]
Dexamethasone	Polymeric nanoparticle [Hyaluronic acid and ethylene diamine]	PLGA	Cell differentiation of mesenchymal stem cells	[101]
Sorafenib	Silica-polymer hybrid nanoparticle	Hydrodynamic flow focusing (HFF)	Antitumor treatment	[96]
Propofol	Liposomes	Herringbone pattern-shaped microfluidic device	Anesthetic agent	[102]
Itraconazole	Polymeric nanoparticle [Poly (methylsilsesquioxane)]	V-junction	Antifungal treatment	[103]
Docetaxel	Polymeric nanoparticle	PLGA-PEG	Anticancer treatment	[104]

PLGA poly(lactic-co-glycolic acid), *HFF* hydrodynamic flow focusing, *PEG* polyethylene glycol

7. **Inorganic and hybrid nanoparticles**
   Microfluidic techniques also facilitate the engineering of nanoformulations constituted of inorganic compounds, viz. metals and their oxides, and silica [94, 95]. Such nanoformulations can be preprogrammed to respond to certain triggers like pH so as to control their release patterns and enable sustained release [96]. Microfluidic techniques enabled PGLA to undergo precipitation with the anticancer drug paclitaxel and iron oxide encapsulated by nanocarriers. This formulation has been used as a treatment option in patients with bone cancer. Such hybrid nanoparticles contain favorable properties like the ability to efficiently target a specific tumor, and have extended retention time in the bloodstream [97]. Similar nanoformulations have also been used as imaging tools and for targeted tumor therapy [98, 99].

   Some nanoparticle-encapsulated drugs fabricated by microfluidics are shown in Table 6.1.

6.6 Advantages of Using Microfluidics in Local Drug Delivery

Microfluidic devices offer certain advantages over conventional DDS in localized drug delivery, which will be listed in this section:

1. **Ability to control rate and pattern of drug delivery**
   Microfluidic devices engineered with minute reservoirs that are integrated with the DDS have facilitated the advent of delivery platforms that can intricately

control the rate of drug delivery [42]. These microreservoirs enable the formulation to be programmed in such a manner that helps control delivery times and greatly enhances stability of the drug in the body [105]. These were previously not possible with traditional DDS. The microreservoirs provide a multitude of options with regard to the patterns of dosing and release of the drug under certain physiological triggers, such as pH, temperature, and magnetic field [22, 23, 106].

2. **Sustained release**
The concentration of a drug in the bloodstream wanes over time owing to several factors, necessitating the repeated administration of the said drug in order to maintain desired therapeutic concentration. To overcome this, microfluidic platforms provide the capacity to control the release pattern of the drug so as to enable sustained release over a longer time point in order to maintain the requisite therapeutic window for the disease in question [107]. Reducing multiple administrations of the drug by extending the circulation time in the bloodstream also helps reduce unwanted systemic adverse effects and potential toxicity. Microfluidic devices also facilitate improved bioavailability of the drug and negate fluctuations in the therapeutic level of the drug within the bloodstream, thus enhancing the efficacy over the entire therapeutic window [107–109].

Sustained release can be achieved by the fundamental process of diffusion. Microreservoirs integrated into the DDS contain the API that is released in the bloodstream upon certain physiological stimuli that dissolve the encapsulation [25, 107, 110]. The material used to encapsulate the microcarrier is of great importance as the dissolution and subsequent release of the payload is dependent on how readily it is degraded by the relevant physiological trigger. The length of the microchannel also plays a pivotal role in the ADME of the drug and can be altered based on how long the drug is required to circulate in the body [111, 112]. Sustained release can also be facilitated by constructing microdevices with multiple such microchannels of varying dimensions that are capable of containing more than one drug with different release patterns and triggers [113]. Drugs that are easily degraded upon entry inside the body can be delivered via microdevices with longer microchannels to help circumvent their poor stability. Microneedles are another microfluidic platform that facilitate sustained release patterns for up to 24 h and have been used to efficiently deliver peptide-based drugs through the skin [114–116].

6.7 Microfluidics for Localized Drug Delivery

Microfluidic platforms have enabled targeted drug delivery with greater efficiency and lower off-target toxicity than conventional DDS. This section will highlight some site-specific targeted drug delivery using microfluidic devices.

6.7.1 Skin

While transdermal patches have long been used to deliver drugs through the skin, it faces several obstacles such as painful penetration and poor permeability of the drug

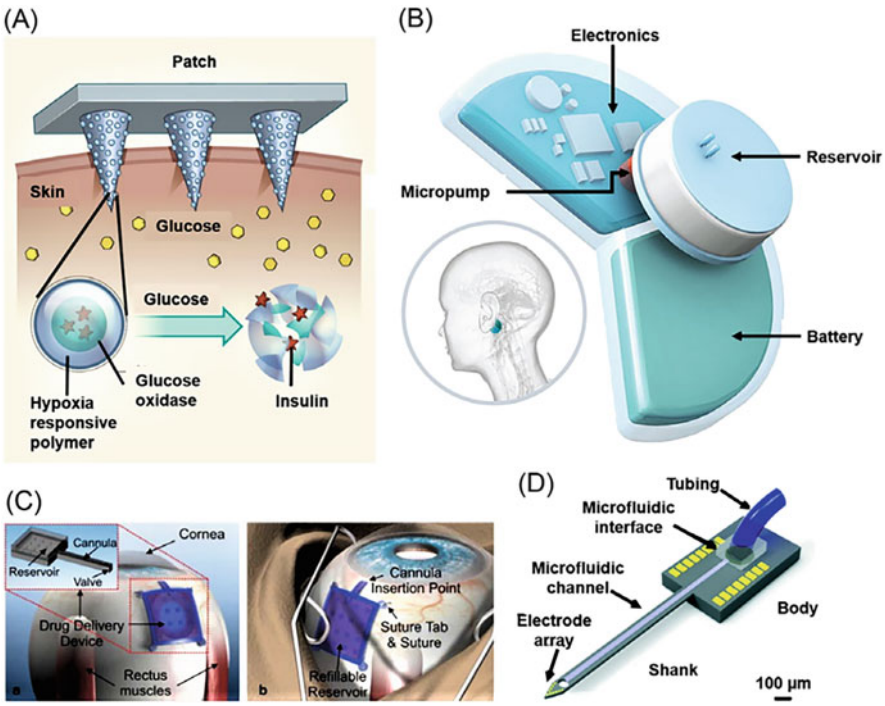

Fig. 6.1 Drug delivery and diagnostics using microfluidic devices. Diagrammatic representation of microfluidic devices that have been used for local drug delivery (**A**) Microneedles used for delivering insulin and monitoring glucose levels in the blood. (**B**) Pictorial depiction of a microfluidic device used for localized drug delivery in the inner ear. (**C**) Illustration of a microfluidic device that can be used for targeted drug delivery in ocular diseases. (**D**) A microfluidic device which is capable of drug delivery to the brain. (Reprinted from *Microfluidic technologies for local drug delivery* https://doi.org/10.1016/B978-0-12-812659-2.00010-7 with an official license from the Elsevier Copyright Clearance Center, Inc. ("CCC"))

due to the barrier posed by the stratum corneum (SC) [117]. Microneedles have been used as a viable alternative as they can help overcome some of these issues. Microneedles (which are up to 2 mm long) can bypass the SC and the underlying nerve fibers and vasculature to efficiently permeate the skin and ensure that the drug reaches the bloodstream [118]. Microneedles help improve patient compliance as they are devoid of the pain and trauma associated with needles and patches that penetrate the dermis. Microneedles can also be fabricated so as to monitor glucose levels in the bloodstream of diabetic patients, as well as release insulin as and when required [119]. This has been depicted in Fig. 6.1A.

They can also be fabricated with polymers that dissolve under certain bodily triggers over a specific period of time so as to enable sustained release and maintain adequate therapeutic concentration in the body. Microneedles can also be subjected to structural modifications based on the disease severity and the requisite release

patterns and therapeutic window. Different structures and shapes, such as solid microneedles, drug-coated microneedles, hollow microneedles, and dissolvable microneedles have been touted for different use-case scenarios [120]. Hollow microneedles, which are typically composed of biodegradable polymers that are readily dissolved under certain triggers, can be incorporated in the DDS both singularly and in multiples. When the need of the hour is continuous drug delivery over an extended time period or sampling of a specific body fluid, multiple hollow microneedles are the go-to-choice as they facilitate the rapid delivery of the drug formulation to more parts of the body. The drug housed in the microreservoir is released under pressure, and the microfluidic device is programmed so as to exert pressure upon entry inside the body. Solid microneedles, on the other hand, contain a sharp needle-like tip that is used to puncture the skin. However, as the nozzle is very small in diameter, the penetration is almost devoid of pain-unlike conventional needles. Solid microneedles are typically made up of polymers or materials like metal and ceramic. Following penetration, the needle can be removed and the drug formulation (typically a patch or a topical formulation such as a gel and cream) is applied over the punctured area. Drug-coated microneedles are similar to solid microneedles, apart from the fact that the needle is coated with the drug over which an outer layering is coated. After permeating the skin, the coating is dissolved and the drug is released, following which the microneedle can be removed [116].

6.7.2 Inner Ear

Owing to the sensitivity and complex physiology of the inner ear, it is one of the hardest locations in the body to access for targeted drug delivery. The blood-cochlea barrier occlusion renders conventional routes of administration ineffective [121]. Potential off-target toxicity caused by injectable medications can further lead to tinnitus as well as loss of hearing and balance. The Organ of Corti, situated within the cochlea, is particularly sensitive to mechanical damage caused by external sources [122, 123]. The human cochlea houses a minute volume of perilymph fluid (approximately 80 μL)—and any minute alterations to this volume can potentially affect the normal functioning of the cochlea [121, 124]. Thus, targeted drug administration at the inner ear is highly challenging for conventional DDS, thereby necessitating the use of microfluidic technologies [125]. Microfluidic devices for targeted drug delivery at the inner ear utilize actuators to provide impulses to the microreservoir at cyclical time-points. The relaxation of the actuator upon receiving the impulse triggers the release of the fluid to the membrane covering the microreservoir. When there is a greater buildup of fluid within the cochlea, the actuator is programmed to alter the pumping mechanism so as to return the excess cochlear fluid to the reservoir. Upon the relaxation of the membrane, the microdevice is replenished with fluid again [121, 126]. These cycles are regulated owing to the control that can be wielded over the microfluidic device used for targeted DDS, and helps provide checks and balances to regulate the volume of drug as well as perilymph within the cochlea. The drug is delivered to the inner ear by the process

of diffusion. As the drug delivery system itself is self-regulating, there is no net flow of fluid either inside or outside the cochlea. When the greater concentration of the drug mixed with the fluid is delivered to the inner ear, the lower-concentration fluid is released back to the microreservoir. This helps prevent a sudden and excess buildup of fluid as it can potentially affect cochlear function and result in alterations in ear pressure, and also helps attain a high but stable therapeutic concentration of the drug inside the ear [121]. Apart from these, research is also underway to develop microfluidic devices engineered like a prosthetic cochlea, so as to achieve sustained release DDS for the inner ear diseases that require a greater therapeutic window [127]. A diagrammatic representation of a microfluidic device used for localized drug delivery in the inner ear has been shown in Fig. 6.1B.

6.7.3 Eye

The complexity and sensitivity of the human eye has made targeted drug delivery really challenging. Most therapeutic options for the eye are based on the topical mode of administration (in the form of eye drops) [128]. Ocular ailments leading to blindness are typically borne out of uveitis and macular degeneration, the onset of which occurs in old age. Glaucoma is another causative factor for blindness all over the globe [129]. As most such ocular ailments are chronic in nature, the therapeutic concentration of the apropos treatment needs to be sufficiently maintained over an extended period [130, 131]. Efficacy of the currently used DDS for ocular diseases are hindered by the anatomical complexity of the eye, sub-par patient compliance owing to repeated topical administration, and potential adverse effects of the drug being used [132, 133]. Difficulty in crossing the corneal membrane, nasolacrimal drainage, and physiological events like blinking and tearing also adversely affect the efficacy and bioavailability of ocular treatment regimens [130]. Single-use contact lenses have been touted as a potentially novel DDS that can help overcome some of these obstacles [134, 135]. Microfluidic devices fabricated with tiny pumps have emerged as a viable option to ensure sustained release of ocular treatments [136, 137]. An illustration of such microfluidic devices has been provided in Fig. 6.1C. These micropumps can also be replenished as and when required, and they can also be refilled with a different drug—thus effectively ensuring the use of a single DDS for the administration of multiple drugs based on the requirement [138]. Microfluidic systems have also been used in the aftermath of corrective eye surgery in patients suffering from glaucoma to prevent fibrosis of the ocular tissue [139].

6.7.4 Brain

The blood-brain barrier has hindered the discovery of novel treatment regimens for neurodegenerative disorders as many potential therapeutic options fail to cross this barrier on systemic administration, ultimately leading to off-target toxicity at other

organs and tissues. Direct delivery of such drugs to the brain can potentially alleviate this limitation. Convection-enhanced delivery (CED), which uses catheters to deliver the drug directly to the brain tissue by bypassing the blood-brain barrier, has been touted as a potentially viable alternative [140, 141]. Many drugs that successfully diffuse across the blood-brain barrier can only traverse short distances before their metabolism by the brain or elimination from interstitial spaces [141–143]. Microfluidic devices fabricated with silicon probes have been used to overcome the same. However, such probes often trigger an immune response from the body akin to that which is triggered by an infectious pathogen [144]. To avoid such a reaction, studies have focused on probes with greater biological compatibility with the underlying tissue [145]. CED has also been utilized in the treatment of glioblastoma to treat brain tumors [141]. Research is also underway to help develop a hybrid DDS composed of CED and ion pumps for targeted drug delivery in neurological diseases [146]. A pictorial depiction of a microfluidic device that is capable of drug delivery to the brain has been provided in Fig. 6.1D.

6.8 Recent Trends in Microfluidic-Mediated Drug Delivery Systems

The previous few years has witnessed a steady rise in novel discoveries pertaining to microfluidic devices used for targeted drug delivery. This section will touch upon a few such advances, with an emphasis on anticancer treatments.

1. **Nanoparticles for delivering antitumor drugs**
   Anticancer drugs such as sorafenib, methotrexate, and paclitaxel have been encapsulated within a single hybrid nanoparticle engineered using a microfluidic platform [147]. Differential sensitivity of the three drugs to physiological triggers like pH, and the different solubility of the materials used to encapsulate the drugs (such as acetal dextran) in solvents such as water and ethanol, is crucial in facilitating combined chemotherapy at different time-points and physiological conditions through a solitary microfluidic DDS. The advent of microfluidic techniques has enabled the fabrication of such coaxial flowing nanoparticles with uniform surface smoothness, greatly improved cellular absorption, and very little variability in dimensions, which have made it possible to combine drugs with different release profiles to provide combinatorial therapy in cancer. Polymeric nanoparticles (constituted of materials like PLGA) fabricated using microfluidic techniques have also been used to encapsulate natural compounds like curcumin to treat tumors [148]. Such nanoparticles, upon reduction in particle dimensions, have successfully demonstrated viability in penetrating mucosal layers to facilitate targeted drug delivery [149].
2. **Nanocapsules for delivering anticancer drugs**
   The microfluidic fabrication of the drug paclitaxel encapsulated by dendritic polyethylene nanoparticles has facilitated the sustained release of the drug for up to 14 days by merely tweaking the flow rate during the engineering of the said

nanocapsule. By altering the duration for which the aqueous phase mixes with the organic phase within the microchannels, the release patterns of the drug can further be changed [150]. The nanoformulation has been successfully studied on a human breast cancer cell line (MCF-7) and it has demonstrated superior anticancer therapeutic effect in comparison to a conventional formulation of paclitaxel.

3. **Nanoliposomes for tumor chemotherapy**
   Nanoliposomes are a novel platform that have been utilized as DDS in various forms of cancer therapy. Nanoliposome encapsulated doxorubicin, an antitumor drug, has been fabricated by microfluidics for the treatment of breast cancer [151]. Studies have demonstrated that greater biodistribution and more efficient targeting of tumors can be achieved by nanoliposome encapsulated drugs as compared to their conventional formulation counterparts. The dimensions of the nanoliposomes can be tweaked to obtain favorable clearance rates—larger nanoliposomes tend to be better distributed to organs such as the liver, while the smaller ones show greater clearance from the bloodstream. Nanoliposomes have also been used to achieve a combinatorial effect when two or more compounds—viz. cisplatin and curcumin—are encapsulated together to penetrate cancer cells better than if they are used individually [152].

Apart from these, microfluidic platforms have also made huge strides in the domains of DNA sequencing, cell sorting, and electrophoresis in recent years [153–155]. Microfluidic platforms have also facilitated the formation of two-pronged devices that can be used simultaneously as a DDS as well as a monitoring system for tracking certain metabolites in the body [126, 156]. Microfluidics has also heralded the recent breakthrough in lab-on-a-chip devices. Such devices are unique as they facilitate research on multifarious domains including drug delivery, synthesis, and development [157–159].

6.9 Industrial Applications

The pharmaceutical industry is one of the biggest sectors in the world with a global market share of more than a trillion dollars [160]. Drug discovery, R&D, drug formulation, and manufacturing, however, require large amounts of investment as well as time. As a result, pharmaceutical enterprises are always on the lookout for novel implementations that can potentially reduce manufacturing costs, time, and raw material wastage [161]. Microfluidic technologies tick all of the aforementioned boxes, and provide a sustainable platform for consistently churning out uniform DDS with much reduced intra and interbatch variability, and greater efficiency with respected to targeted drug delivery. Microfluidic platforms also enable scaling up of drug manufacturing to industrial levels in compliance with GMP [162]. This can be achieved by increasing the cumulative fluid velocity inside the microchannels by simply utilizing a greater number of microfluidic devices with the same fluid velocity [158, 163]. Manufacturers can also use several micropumps for each microfluidic

device so as to provide multiple pressure sources—this facilitates the production of nanoparticles with greater uniformity in shape, size, and structure, albeit at a greater cost [13, 164, 165]. Microfluidic devices have been used in the past few years to usher in the onset of nanoassembler GMP as a viable option for industrial-scale production of drugs [162]. Such new-age modifications can potentially play a crucial role in how nanomedicines are produced on a global scale in the near future.

6.10 Concluding Remarks

The steady rise of microfluidic technologies over the past few decades has been a breath of fresh air for the pharmaceutical sector as it has enabled the industrial scale production of miniaturized devices for targeted drug delivery and gene delivery. Microfluidic devices also have significant clinical relevance owing to their structural uniformity, reproducibility, and highly controllable nature—properties that have facilitated novel DDS with greater stability, efficiency, and sustained release patterns. Microreservoirs that can encapsulate multiple drugs with different release kinetics have even made it possible to deliver multiple drugs through a single DDS, thereby greatly improving patient compliance. Such DDS release their contents under different physiological stimuli (viz. temperature, pH, and enzymatic activity) based on the properties of the material used to encapsulate the microdevice, as well as that of the drug itself. The ability to fine-tune the release patterns and increase retention times has also reduced off-target toxicity and the need for multiple administrations, which is particularly helpful in chronic debilitating illnesses such as cancer. The upscaling of nanoformulations to levels that have hitherto not been achieved has also been made possible by the advent of microfluidic technologies.

6.11 Future Perspectives

While microfluidic technologies are already being used for targeted drug delivery, their range of applications in the future can be wide-ranging and serve several therapeutic as well as diagnostic needs that are presently unmet. Long-term stability and biological compatibility of microfluidic devices both in vivo and outside need to be further studied to enable the fabrication of DDS that can potentially be implanted within the body for far longer (in chronic illnesses) than the currently available treatment modalities. Materials such as bioink—that can alter the physical properties and dimensions of microdevices, can also potentially be used to fabricate nanoformulations capable of altering themselves in response to physiological stimuli [166]. Personalized microfluidic DDS tailor-made to suit an individual's genetic and physiological conditions should also be researched upon to further reduce possibilities of systemic and off-target toxicities. 3D printing, which currently facilitates platforms such as cell-on-a-chip and organ-on-a-chip, can also be further improved upon to fabricate in vitro microfluidic chips that are capable of testing a

drug's efficacy, release kinetics, and potential toxic effects without the need for preclinical or clinical studies [167–169].

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References

1. Dmochowski RR, Staskin DR (2002) Advances in drug delivery: improved bioavailability and drug effect. Curr Urol Rep 3(6):439–444. https://doi.org/10.1007/s11934-002-0094-4
2. Coelho JF, Ferreira PC, Alves P, Cordeiro R, Fonseca AC, Góis JR, Gil MH (2010) Drug delivery systems: advanced technologies potentially applicable in personalized treatments. EPMA J 1(1):164–209. https://doi.org/10.1007/s13167-010-0001-x
3. Oliveira KA, Silva PB Me, de Souza FR, Martins FT, Coltro WKT (2014) Kinetic study of glucose oxidase on microfluidic toner-based analytical devices for clinical diagnostics with image-based detection. Anal Methods 6(14):4995–5000. https://doi.org/10.1039/C4AY00260A
4. Puigmartí-Luis J (2014) Microfluidic platforms: a mainstream technology for the preparation of crystals. Chem Soc Rev 43(7):2253–2271. https://doi.org/10.1039/c3cs60372e
5. Samiei E, Hoorfar M (2015) Systematic analysis of geometrical based unequal droplet splitting in digital microfluidics. J Micromech Microeng 25(5):055008. https://doi.org/10.1088/0960-1317/25/5/055008
6. Zhu Y, Fang Q (2013) Analytical detection techniques for droplet microfluidics—a review. Anal Chim Acta 787:24–35. https://doi.org/10.1016/j.aca.2013.04.064
7. Ahn J, Ko J, Lee S, Yu J, Kim Y, Jeon NL (2018) Microfluidics in nanoparticle drug delivery; from synthesis to pre-clinical screening. Adv Drug Deliv Rev 128:29–53. https://doi.org/10.1016/j.addr.2018.04.001
8. Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S, Habtemariam S, Shin H-S (2018) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 16(1):71. https://doi.org/10.1186/s12951-018-0392-8
9. Valencia PM, Farokhzad OC, Karnik R, Langer R (2012) Microfluidic technologies for accelerating the clinical translation of nanoparticles. Nat Nanotechnol 7(10):623–629. https://doi.org/10.1038/nnano.2012.168
10. Wang J, Mao W, Lock LL, Tang J, Sui M, Sun W, Cui H, Xu D, Shen Y (2015b) The role of micelle size in tumor accumulation, penetration, and treatment. ACS Nano 9(7):7195–7206. https://doi.org/10.1021/acsnano.5b02017
11. Mo R, Jiang T, Di J, Tai W, Gu Z (2014) Emerging micro- and nanotechnology based synthetic approaches for insulin delivery. Chem Soc Rev 43(10):3595–3629. https://doi.org/10.1039/c3cs60436e
12. Athari SS, Pourpak Z, Folkerts G, Garssen J, Moin M, Adcock IM, Movassaghi M, Ardestani MS, Moazzeni SM, Mortaz E (2016) Conjugated Alpha-Alumina nanoparticle with vasoactive intestinal peptide as a Nano-drug in treatment of allergic asthma in mice. Eur J Pharmacol 791:811–820. https://doi.org/10.1016/j.ejphar.2016.10.014
13. Capretto L, Mazzitelli S, Brognara E, Lampronti I, Carugo D, Hill M, Zhang X, Gambari R, Nastruzzi C (2012) Mithramycin encapsulated in polymeric micelles by microfluidic technology as novel therapeutic protocol for beta-thalassemia. Int J Nanomedicine 7:307–324. https://doi.org/10.2147/IJN.S25657

14. Matoba T, Koga J-I, Nakano K, Egashira K, Tsutsui H (2017) Nanoparticle-mediated drug delivery system for atherosclerotic cardiovascular disease. J Cardiol 70(3):206–211. https://doi.org/10.1016/j.jjcc.2017.03.005
15. Sanjay ST, Zhou W, Dou M, Tavakoli H, Ma L, Xu F, Li X (2018) Recent advances of controlled drug delivery using microfluidic platforms. Adv Drug Deliv Rev 128:3–28. https://doi.org/10.1016/j.addr.2017.09.013
16. Hui Y, Yi X, Hou F, Wibowo D, Zhang F, Zhao D, Gao H, Zhao C-X (2019) Role of nanoparticle mechanical properties in cancer drug delivery. ACS Nano 13(7):7410–7424. https://doi.org/10.1021/acsnano.9b03924
17. Su C, Liu Y, Li R, Wu W, Fawcett JP, Gu J (2019) Absorption, distribution, metabolism and excretion of the biomaterials used in nanocarrier drug delivery systems. Adv Drug Deliv Rev 143:97–114. https://doi.org/10.1016/j.addr.2019.06.008
18. Park JH, Saravanakumar G, Kim K, Kwon IC (2010a) Targeted delivery of low molecular drugs using chitosan and its derivatives. Adv Drug Deliv Rev 62(1):28–41. https://doi.org/10.1016/j.addr.2009.10.003
19. Sanjay ST, Dou M, Fu G, Xu F, Li X (2017) Controlled drug delivery using microdevices. Curr Pharm Biotechnol 17(9):772–787
20. Rowland M (1972) Influence of route of administration on drug availability. J Pharm Sci 61(1):70–74. https://doi.org/10.1002/jps.2600610111
21. Theeuwes F, Yum SI, Haak R, Wong P (1991) Systems for triggered, pulsed, and programmed drug delivery. Ann N Y Acad Sci 618(1):428–440. https://doi.org/10.1111/j.1749-6632.1991.tb27262.x
22. Hatakeyama H (2017) Recent advances in endogenous and exogenous stimuli-responsive nanocarriers for drug delivery and therapeutics. Chem Pharm Bull 65(7):612–617. https://doi.org/10.1248/cpb.c17-00068
23. Liu M, Du H, Zhang W, Zhai G (2017c) Internal stimuli-responsive nanocarriers for drug delivery: design strategies and applications. Mater Sci Eng C 71:1267–1280. https://doi.org/10.1016/j.msec.2016.11.030
24. Wen H, Jung H, Li X (2015) Drug delivery approaches in addressing clinical pharmacology-related issues: opportunities and challenges. AAPS J 17(6):1327–1340. https://doi.org/10.1208/s12248-015-9814-9
25. Zafar Razzacki S, Thwar PK, Yang M, Ugaz VM, Burns MA (2004) Integrated microsystems for controlled drug delivery. Adv Drug Deliv Rev 56(2):185–198. https://doi.org/10.1016/j.addr.2003.08.012
26. McGee BJ, Kennedy DR, Walker GC (1970) Some factors affecting release and availability of drugs from hard gelatin capsules. J Pharm Sci 59(10):1430–1433. https://doi.org/10.1002/jps.2600591013
27. Wood JH (1965) In vivo drug release rate from hard gelatin capsules. J Pharm Sci 54(8):1207–1208. https://doi.org/10.1002/jps.2600540827
28. Kang J-S, Lee M-H (2009) Overview of therapeutic drug monitoring. Korean J Intern Med 24(1):1–10. https://doi.org/10.3904/kjim.2009.24.1.1
29. Greenblatt DJ (1985) Elimination half-life of drugs: value and limitations. Annu Rev Med 36(1):421–427. https://doi.org/10.1146/annurev.me.36.020185.002225
30. Coleman MD (2010) Factors affecting drug metabolism. In: Human drug metabolism. Wiley, Hoboken, NJ, pp 159–212. https://doi.org/10.1002/9780470689332.ch7
31. Thomas JN, Guhan SS, Pettit DK (2010) Process development of protein therapeutics. In: Burger's medicinal chemistry and drug discovery. Wiley, Hoboken, NJ, pp 289–344. https://doi.org/10.1002/0471266949.bmc160
32. Torchilin VP (2000) Drug targeting. Eur J Pharm Sci 11:S81–S91. https://doi.org/10.1016/S0928-0987(00)00166-4
33. Mills JK, Needham D (1999) Targeted drug delivery. Expert Opin Ther Pat 9(11):1499–1513. https://doi.org/10.1517/13543776.9.11.1499

34. Bae YH, Park K (2011) Targeted drug delivery to tumors: myths, reality and possibility. J Control Release 153(3):198–205. https://doi.org/10.1016/j.jconrel.2011.06.001
35. Srinivasarao M, Low PS (2017) Ligand-targeted drug delivery. Chem Rev 117(19): 12133–12164. https://doi.org/10.1021/acs.chemrev.7b00013
36. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM (2016) PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Adv Drug Deliv Rev 99:28–51. https://doi.org/10.1016/j.addr.2015.09.012
37. Wang J, Ren L, Li L, Liu W, Zhou J, Yu W, Tong D, Chen S (2009) Microfluidics: a new cosset for neurobiology. Lab Chip 9(5):644–652. https://doi.org/10.1039/b813495b
38. Whitesides GM (2006) The origins and the future of microfluidics. Nature 442(7101): 368–373. https://doi.org/10.1038/nature05058
39. Jones CN, Hoang AN, Dimisko L, Hamza B, Martel J, Irimia D (2014) Microfluidic platform for measuring neutrophil chemotaxis from unprocessed whole blood. J Vis Exp 88:51215. https://doi.org/10.3791/51215
40. Li XJ, Valadez AV, Zuo P, Nie Z (2012) Microfluidic 3D cell culture: potential application for tissue-based bioassays. Bioanalysis 4(12):1509–1525. https://doi.org/10.4155/bio.12.133
41. Liu D, Zhang H, Fontana F, Hirvonen JT, Santos HA (2017b) Microfluidic-assisted fabrication of carriers for controlled drug delivery. Lab Chip 17(11):1856–1883. https://doi.org/10.1039/C7LC00242D
42. Riahi R, Tamayol A, Shaegh SAM, Ghaemmaghami AM, Dokmeci MR, Khademhosseini A (2015) Microfluidics for advanced drug delivery systems. Curr Opin Chem Eng 7:101–112. https://doi.org/10.1016/j.coche.2014.12.001
43. Yu B, Lee RJ, Lee LJ (2009) Chapter 7—Microfluidic methods for production of liposomes. In: Methods in enzymology, vol 465. Academic, London, pp 129–141. https://doi.org/10.1016/S0076-6879(09)65007-2
44. Duncanson WJ, Lin T, Abate AR, Seiffert S, Shah RK, Weitz DA (2012) Microfluidic synthesis of advanced microparticles for encapsulation and controlled release. Lab Chip 12(12):2135–2145. https://doi.org/10.1039/C2LC21164E
45. Zhao C-X (2013) Multiphase flow microfluidics for the production of single or multiple emulsions for drug delivery. Adv Drug Deliv Rev 65(11):1420–1446. https://doi.org/10.1016/j.addr.2013.05.009
46. Huang X, Lee RJ, Qi Y, Li Y, Lu J, Meng Q, Teng L, Xie J (2017) Microfluidic hydrodynamic focusing synthesis of polymer-lipid nanoparticles for siRNA delivery. Oncotarget 8(57): 96826–96836. https://doi.org/10.18632/oncotarget.18281
47. Chen G, Roy I, Yang C, Prasad PN (2016) Nanochemistry and nanomedicine for nanoparticle-based diagnostics and therapy. Chem Rev 116(5):2826–2885. https://doi.org/10.1021/acs.chemrev.5b00148
48. Sebastian V, Arruebo M (2019) Chapter 8—Microfluidic production of inorganic nanomaterials for biomedical applications. In: Santos HA, Liu D, Zhang H (eds) Microfluidics for pharmaceutical applications. William Andrew Publishing, Norwich, NY, pp 179–216. https://doi.org/10.1016/B978-0-12-812659-2.00008-9
49. Kodzius R, Xiao K, Wu J, Yi X, Gong X, Foulds IG, Wen W (2012) Inhibitory effect of common microfluidic materials on PCR outcome. Sensors Actuators B Chem 161(1):349–358. https://doi.org/10.1016/j.snb.2011.10.044
50. Park J, Li J, Han A (2010b) Micro-macro hybrid soft-lithography master (MMHSM) fabrication for lab-on-a-chip applications. Biomed Microdevices 12(2):345–351. https://doi.org/10.1007/s10544-009-9390-9
51. Lee WS, Jambovane S, Kim D, Hong JW (2009) Predictive model on micro droplet generation through mechanical cutting. Microfluid Nanofluid 7(3):431. https://doi.org/10.1007/s10404-009-0412-y
52. Nunes PS, Ohlsson PD, Ordeig O, Kutter JP (2010) Cyclic olefin polymers: emerging materials for lab-on-a-chip applications. Microfluid Nanofluid 9(2):145–161. https://doi.org/10.1007/s10404-010-0605-4

53. Kinnear C, Moore TL, Rodriguez-Lorenzo L, Rothen-Rutishauser B, Petri-Fink A (2017) Form follows function: nanoparticle shape and its implications for nanomedicine. Chem Rev 117(17):11476–11521. https://doi.org/10.1021/acs.chemrev.7b00194
54. Beck-Broichsitter M, Nicolas J, Couvreur P (2015) Design attributes of long-circulating polymeric drug delivery vehicles. Eur J Pharm Biopharm 97(Pt B):304–317. https://doi.org/10.1016/j.ejpb.2015.03.033
55. Yokel RA (2016) Physicochemical properties of engineered nanomaterials that influence their nervous system distribution and effects. Nanomedicine 12(7):2081–2093. https://doi.org/10.1016/j.nano.2016.05.007
56. Feng Q, Sun J, Jiang X (2016) Microfluidics-mediated assembly of functional nanoparticles for cancer-related pharmaceutical applications. Nanoscale 8(25):12430–12443. https://doi.org/10.1039/C5NR07964K
57. Wang J, Chen W, Sun J, Liu C, Yin Q, Zhang L, Xianyu Y, Shi X, Hu G, Jiang X (2014) A microfluidic tubing method and its application for controlled synthesis of polymeric nanoparticles. Lab Chip 14(10):1673–1677. https://doi.org/10.1039/C4LC00080C
58. Belliveau NM, Huft J, Lin PJ, Chen S, Leung AK, Leaver TJ, Wild AW, Lee JB, Taylor RJ, Tam YK, Hansen CL, Cullis PR (2012) Microfluidic synthesis of highly potent limit-size lipid nanoparticles for in vivo delivery of siRNA. Mol Ther Nucleic Acids 1(8):e37. https://doi.org/10.1038/mtna.2012.28
59. He Y, Park K (2016) Effects of the microparticle shape on cellular uptake. Mol Pharm 13(7):2164–2171. https://doi.org/10.1021/acs.molpharmaceut.5b00992
60. Liu D, Bernuz CR, Fan J, Li W, Correia A, Hirvonen J, Santos HA (2017a) A nano-in-nano vector: merging the best of polymeric nanoparticles and drug nanocrystals. Adv Funct Mater 27(9):1604508. https://doi.org/10.1002/adfm.201604508
61. Dashtimoghadam E, Fahimipour F, Tongas N, Tayebi L (2020) Microfluidic fabrication of microcarriers with sequential delivery of VEGF and BMP-2 for bone regeneration. Sci Rep 10(1):11764. https://doi.org/10.1038/s41598-020-68221-w
62. Ran R, Sun Q, Baby T, Wibowo D, Middelberg APJ, Zhao C-X (2017) Multiphase microfluidic synthesis of micro- and nanostructures for pharmaceutical applications. Chem Eng Sci 169:78–96. https://doi.org/10.1016/j.ces.2017.01.008
63. Santo RD, Digiacomo L, Palchetti S, Palmieri V, Perini G, Pozzi D, Papi M, Caracciolo G (2019) Microfluidic manufacturing of surface-functionalized graphene oxide nanoflakes for gene delivery. Nanoscale 11(6):2733–2741. https://doi.org/10.1039/C8NR09245A
64. Lee TY, Choi TM, Shim TS, Frijns RAM, Kim S-H (2016) Microfluidic production of multiple emulsions and functional microcapsules. Lab Chip 16(18):3415–3440. https://doi.org/10.1039/C6LC00809G
65. Clegg PS, Tavacoli JW, Wilde PJ (2016) One-step production of multiple emulsions: microfluidic, polymer-stabilized and particle-stabilized approaches. Soft Matter 12(4):998–1008. https://doi.org/10.1039/C5SM01663K
66. Sapei L, Naqvi MA, Rousseau D (2012) Stability and release properties of double emulsions for food applications. Food Hydrocoll 27(2):316–323. https://doi.org/10.1016/j.foodhyd.2011.10.008
67. Jo YK, Lee D (2020) Biopolymer microparticles prepared by microfluidics for biomedical applications. Small 16(9):1903736. https://doi.org/10.1002/smll.201903736
68. Michelon M, Huang Y, de la Torre LG, Weitz DA, Cunha RL (2019) Single-step microfluidic production of W/O/W double emulsions as templates for β-carotene-loaded giant liposomes formation. Chem Eng J 366:27–32. https://doi.org/10.1016/j.cej.2019.02.021
69. Moros M, Mitchell SG, Grazu V, de la Fuente JM (2013) The fate of nanocarriers as nanomedicines in vivo: important considerations and biological barriers to overcome. Curr Med Chem 20(22):2759–2778. https://www.eurekaselect.com/article/52620. Accessed 10 Jan 2022

70. Tomeh MA, Hadianamrei R, Zhao X (2019) Silk fibroin as a functional biomaterial for drug and gene delivery. Pharmaceutics 11(10):494. https://doi.org/10.3390/pharmaceutics11100494
71. Wang W (2015) Advanced protein formulations. Protein Sci 24(7):1031–1039. https://doi.org/10.1002/pro.2684
72. Bose RJ, Paulmurugan R, Moon J, Lee S-H, Park H (2018) Cell membrane-coated nanocarriers: the emerging targeted delivery system for cancer theranostics. Drug Discov Today 23(4):891–899. https://doi.org/10.1016/j.drudis.2018.02.001
73. Kim Y, Fay F, Cormode DP, Sanchez-Gaytan BL, Tang J, Hennessy EJ, Ma M, Moore K, Farokhzad OC, Fisher EA, Mulder WJM, Langer R, Fayad ZA (2013) Single step reconstitution of multifunctional high-density lipoprotein-derived nanomaterials using microfluidics. ACS Nano 7(11):9975–9983. https://doi.org/10.1021/nn4039063
74. Molinaro R, Evangelopoulos M, Hoffman JR, Corbo C, Taraballi F, Martinez JO, Hartman KA, Cosco D, Costa G, Romeo I, Sherman M, Paolino D, Alcaro S, Tasciotti E (2018) Design and development of biomimetic nanovesicles using a microfluidic approach. Adv Mater 30(15):e1702749. https://doi.org/10.1002/adma.201702749
75. Grimaldi N, Andrade F, Segovia N, Ferrer-Tasies L, Sala S, Veciana J, Ventosa N (2016) Lipid-based nanovesicles for nanomedicine. Chem Soc Rev 45(23):6520–6545. https://doi.org/10.1039/C6CS00409A
76. Maeki M, Kimura N, Sato Y, Harashima H, Tokeshi M (2018) Advances in microfluidics for lipid nanoparticles and extracellular vesicles and applications in drug delivery systems. Adv Drug Deliv Rev 128:84–100. https://doi.org/10.1016/j.addr.2018.03.008
77. Cheng X, Lee RJ (2016) The role of helper lipids in lipid nanoparticles (LNPs) designed for oligonucleotide delivery. Adv Drug Deliv Rev 99:129–137. https://doi.org/10.1016/j.addr.2016.01.022
78. Wang H, Jiang Y, Peng H, Chen Y, Zhu P, Huang Y (2015a) Recent progress in microRNA delivery for cancer therapy by non-viral synthetic vectors. Adv Drug Deliv Rev 81:142–160. https://doi.org/10.1016/j.addr.2014.10.031
79. Dawidczyk CM, Kim C, Park JH, Russell LM, Lee KH, Pomper MG, Searson PC (2014) State-of-the-art in design rules for drug delivery platforms: lessons learned from FDA-approved nanomedicines. J Control Release 187:133–144. https://doi.org/10.1016/j.jconrel.2014.05.036
80. Mizuno M, Toyota T, Konishi M, Kageyama Y, Yamada M, Seki M (2015) Formation of monodisperse hierarchical lipid particles utilizing microfluidic droplets in a nonequilibrium state. Langmuir 31(8):2334–2341. https://doi.org/10.1021/acs.langmuir.5b00043
81. Kitazoe K, Wang J, Kaji N, Okamoto Y, Tokeshi M, Kogure K, Harashima H, Baba Y (2011) A touch-and-go lipid wrapping technique in microfluidic channels for rapid fabrication of multifunctional envelope-type gene delivery nanodevices. Lab Chip 11(19):3256–3262. https://doi.org/10.1039/C1LC20392D
82. Nakamura T, Kawai M, Sato Y, Maeki M, Tokeshi M, Harashima H (2020) The effect of size and charge of lipid nanoparticles prepared by microfluidic mixing on their lymph node transitivity and distribution. Mol Pharm 17(3):944–953. https://doi.org/10.1021/acs.molpharmaceut.9b01182
83. Zhang H, Tumarkin E, Sullan RMA, Walker GC, Kumacheva E (2007) Exploring microfluidic routes to microgels of biological polymers. Macromol Rapid Commun 28(5):527–538. https://doi.org/10.1002/marc.200600776
84. Gunduz O, Ahmad Z, Stride E, Edirisinghe M (2013) Continuous generation of ethyl cellulose drug delivery nanocarriers from microbubbles. Pharm Res 30(1):225–237. https://doi.org/10.1007/s11095-012-0865-7
85. Valencia PM, Pridgen EM, Rhee M, Langer R, Farokhzad OC, Karnik R (2013) Microfluidic platform for combinatorial synthesis and optimization of targeted nanoparticles for cancer therapy. ACS Nano 7(12):10671–10680. https://doi.org/10.1021/nn403370e

86. Hu Y, Wang Q, Wang J, Zhu J, Wang H, Yang Y (2012) Shape controllable microgel particles prepared by microfluidic combining external ionic crosslinking. Biomicrofluidics 6(2): 026502. https://doi.org/10.1063/1.4720396
87. Koh CG, Kang X, Xie Y, Fei Z, Guan J, Yu B, Zhang X, Lee LJ (2009) Delivery of polyethylenimine/DNA complexes assembled in a microfluidics device. Mol Pharm 6(5): 1333–1342. https://doi.org/10.1021/mp900016q
88. Araújo F, Shrestha N, Shahbazi M-A, Liu D, Herranz-Blanco B, Mäkilä EM, Salonen JJ, Hirvonen JT, Granja PL, Sarmento B, Santos HA (2015) Microfluidic assembly of a multifunctional tailorable composite system designed for site specific combined oral delivery of peptide drugs. ACS Nano 9(8):8291–8302. https://doi.org/10.1021/acsnano.5b02762
89. Bokare A, Takami A, Kim JH, Dong A, Chen A, Valerio R, Gunn S, Erogbogbo F (2019) Herringbone-patterned 3D-printed devices as alternatives to microfluidics for reproducible production of lipid polymer hybrid nanoparticles. ACS Omega 4(3):4650–4657. https://doi.org/10.1021/acsomega.9b00128
90. Valencia PM, Basto PA, Zhang L, Rhee M, Langer R, Farokhzad OC, Karnik R (2010) Single-step assembly of homogenous lipid-polymeric and lipid-quantum dot nanoparticles enabled by microfluidic rapid mixing. ACS Nano 4(3):1671–1679. https://doi.org/10.1021/nn901433u
91. Mieszawska AJ, Kim Y, Gianella A, van Rooy I, Priem B, Labarre MP, Ozcan C, Cormode DP, Petrov A, Langer R, Farokhzad OC, Fayad ZA, Mulder WJM (2013) Synthesis of polymer-lipid nanoparticles for image-guided delivery of dual modality therapy. Bioconjug Chem 24(9):1429–1434. https://doi.org/10.1021/bc400166j
92. Le NHA, Van Phan H, Yu J, Chan H-K, Neild A, Alan T (2018) Acoustically enhanced microfluidic mixer to synthesize highly uniform nanodrugs without the addition of stabilizers. Int J Nanomedicine 13:1353–1359. https://doi.org/10.2147/IJN.S153805
93. Amstad E, Gopinadhan M, Holtze C, Osuji CO, Brenner MP, Spaepen F, Weitz DA (2015) NANOPARTICLES. Production of amorphous nanoparticles by supersonic spray-drying with a microfluidic nebulator. Science (New York, NY) 349(6251):956–960. https://doi.org/10.1126/science.aac9582
94. Al-Ahmady ZS, Donno R, Gennari A, Prestat E, Marotta R, Mironov A, Newman L, Lawrence MJ, Tirelli N, Ashford M, Kostarelos K (2019) Enhanced intraliposomal metallic nanoparticle payload capacity using microfluidic-assisted self-assembly. Langmuir 35(41):13318–13331. https://doi.org/10.1021/acs.langmuir.9b00579
95. Ding S, Attia MF, Wallyn J, Taddei C, Serra CA, Anton N, Kassem M, Schmutz M, Er-Rafik M, Messaddeq N, Collard A, Yu W, Giordano M, Vandamme TF (2018) Microfluidic-assisted production of size-controlled superparamagnetic iron oxide nanoparticles-loaded poly(methyl methacrylate) nanohybrids. Langmuir 34(5):1981–1991. https://doi.org/10.1021/acs.langmuir.7b01928
96. Herranz-Blanco B, Liu D, Mäkilä E, Shahbazi M-A, Ginestar E, Zhang H, Aseyev V, Balasubramanian V, Salonen J, Hirvonen J, Santos HA (2015) On-chip self-assembly of a smart hybrid nanocomposite for antitumoral applications. Adv Funct Mater 25(10): 1488–1497. https://doi.org/10.1002/adfm.201404122
97. Hasani-Sadrabadi MM, Dashtimoghadam E, Bahlakeh G, Majedi FS, Keshvari H, Van Dersarl JJ, Bertsch A, Panahifar A, Renaud P, Tayebi L, Mahmoudi M, Jacob KI (2015) On-chip synthesis of fine-tuned bone-seeking hybrid nanoparticles. Nanomedicine (Lond) 10(23): 3431–3449. https://doi.org/10.2217/nnm.15.162
98. Bleul R, Thiermann R, Marten GU, House MJ, Pierre TGS, Häfeli UO, Maskos M (2013) Continuously manufactured magnetic polymersomes – a versatile tool (not only) for targeted cancer therapy. Nanoscale 5(23):11385–11393. https://doi.org/10.1039/C3NR02190D
99. Cheheltani R, Ezzibdeh RM, Chhour P, Pulaparthi K, Kim J, Jurcova M, Hsu JC, Blundell C, Litt HI, Ferrari VA, Allcock HR, Sehgal CM, Cormode DP (2016) Tunable, biodegradable gold nanoparticles as contrast agents for computed tomography and photoacoustic imaging. Biomaterials 102:87–97. https://doi.org/10.1016/j.biomaterials.2016.06.015

100. Kim M-K, Kim MA, Jenjob R, Lee D-H, Yang S-G (2016) Capillary microfluidics-derived doxorubicin-containing human serum albumin microbeads for transarterial chemoembolization of hepatic cancer. Mater Sci Eng C Mater Biol Appl 62:391–397. https://doi.org/10.1016/j.msec.2016.01.073
101. Parra Saldivar R (2018) Microfluidics technology for drug delivery: a review. Front Biosci 10(1):74–91. https://doi.org/10.2741/e809
102. Kastner E, Verma V, Lowry D, Perrie Y (2015) Microfluidic-controlled manufacture of liposomes for the solubilisation of a poorly water soluble drug. Int J Pharm 485(1–2): 122–130. https://doi.org/10.1016/j.ijpharm.2015.02.063
103. Kucuk I, Ahmad Z, Edirisinghe M, Orlu-Gul M (2014) Utilization of microfluidic V-junction device to prepare surface itraconazole adsorbed nanospheres. Int J Pharm 472(1):339–346. https://doi.org/10.1016/j.ijpharm.2014.06.023
104. Lim J-M, Swami A, Gilson LM, Chopra S, Choi S, Wu J, Langer R, Karnik R, Farokhzad OC (2014) Ultra-high throughput synthesis of nanoparticles with homogeneous size distribution using a coaxial turbulent jet mixer. ACS Nano 8(6):6056–6065. https://doi.org/10.1021/nn501371n
105. Tiwari G, Tiwari R, Sriwastawa B, Bhati L, Pandey S, Pandey P, Bannerjee SK (2012) Drug delivery systems: an updated review. Int J Pharm Invest 2(1):2–11. https://www.jpionline.org/index.php/ijpi/article/view/58
106. Stevenson CL, Santini JT, Langer R (2012) Reservoir-based drug delivery systems utilizing microtechnology. Adv Drug Deliv Rev 64(14):1590–1602. https://doi.org/10.1016/j.addr.2012.02.005
107. Kumar K, Bhowmik D, Srivastava S, Paswan S, Dutta A (2012) Sustained release drug delivery system potential. Pharma Innov 1(2):48–60
108. Jain D, Raturi R, Jain V, Bansal P, Singh R (2011) Recent technologies in pulsatile drug delivery systems. Biomatter 1(1):57–65. https://doi.org/10.4161/biom.1.1.17717
109. Liechty WB, Kryscio DR, Slaughter BV, Peppas NA (2010) Polymers for drug delivery systems. Annu Rev Chem Biomol Eng 1(1):149–173. https://doi.org/10.1146/annurev-chembioeng-073009-100847
110. Nagai N, Kaji H, Onami H, Katsukura Y, Ishikawa Y, Nezhad ZK, Sampei K, Iwata S, Ito S, Nishizawa M, Nakazawa T, Osumi N, Mashima Y, Abe T (2014) A platform for controlled dual-drug delivery to the retina: protective effects against light-induced retinal damage in rats. Adv Healthc Mater 3(10):1555–1560. https://doi.org/10.1002/adhm.201400114
111. Singh TRR, Mcmillan H, Mooney K, Alkilani AZ, Donnelly RF (2013) 6—Microneedles for drug delivery and monitoring. In: James Li X, Zhou Y (eds) Microfluidic devices for biomedical applications. Woodhead Publishing, Sawston, UK, pp 185–230. https://doi.org/10.1533/9780857097040.2.185
112. Valeh-e-Sheyda P, Rahimi M, Parsamoghadam A, Adibi H (2015) Effect of microchannel confluence angles on size reduction of curcumin nano-suspension via liquid anti-solvent precipitation process. J Taiwan Inst Chem Eng 46:65–73. https://doi.org/10.1016/j.jtice.2014.09.024
113. Lee SH, Park M, Park CG, Lee JE, Prausnitz MR, Choy YB (2012) Microchip for sustained drug delivery by diffusion through microchannels. AAPS PharmSciTech 13(1):211–217. https://doi.org/10.1208/s12249-011-9743-6
114. Bariya SH, Gohel MC, Mehta TA, Sharma OP (2012) Microneedles: an emerging transdermal drug delivery system. J Pharm Pharmacol 64(1):11–29. https://doi.org/10.1111/j.2042-7158.2011.01369.x
115. Vinayakumar KB, Hegde GM, Ramachandra SG, Nayak MM, Dinesh NS, Rajanna K (2015) Development of cup shaped microneedle array for transdermal drug delivery. Biointerphases 10(2):021008. https://doi.org/10.1116/1.4919779
116. Wang M, Hu L, Xu C (2017) Recent advances in the design of polymeric microneedles for transdermal drug delivery and biosensing. Lab Chip 17(8):1373–1387. https://doi.org/10.1039/C7LC00016B

117. Sanjay ST, Dou M, Sun J, Li X (2016) A paper/polymer hybrid microfluidic microplate for rapid quantitative detection of multiple disease biomarkers. Sci Rep 6(1):30474. https://doi.org/10.1038/srep30474
118. Tuan-Mahmood T-M, McCrudden MTC, Torrisi BM, McAlister E, Garland MJ, Singh TRR, Donnelly RF (2013) Microneedles for intradermal and transdermal drug delivery. Eur J Pharm Sci 50(5):623–637. https://doi.org/10.1016/j.ejps.2013.05.005
119. Veiseh O, Langer R (2015) A smart insulin patch. Nature 524(7563):39–40. https://doi.org/10.1038/524039a
120. Kim Y-C, Park J-H, Prausnitz MR (2012) Microneedles for drug and vaccine delivery. Adv Drug Deliv Rev 64(14):1547–1568. https://doi.org/10.1016/j.addr.2012.04.005
121. Pararas EEL, Borkholder DA, Borenstein JT (2012) Microsystems technologies for drug delivery to the inner ear. Adv Drug Deliv Rev 64(14):1650–1660. https://doi.org/10.1016/j.addr.2012.02.004
122. Ashmore J (2008) Cochlear outer hair cell motility. Physiol Rev 88(1):173–210. https://doi.org/10.1152/physrev.00044.2006
123. Hudspeth AJ (2014) Integrating the active process of hair cells with cochlear function. Nat Rev Neurosci 15(9):600–614. https://doi.org/10.1038/nrn3786
124. Swan EEL, Mescher MJ, Sewell WF, Tao SL, Borenstein JT (2008) Inner ear drug delivery for auditory applications. Adv Drug Deliv Rev 60(15):1583–1599. https://doi.org/10.1016/j.addr.2008.08.001
125. McCall AA, Swan EEL, Borenstein JT, Sewell WF, Kujawa SG, McKenna MJ (2010) Drug delivery for treatment of inner ear disease: current state of knowledge. Ear Hear 31(2):156–165. https://doi.org/10.1097/AUD.0b013e3181c351f2
126. Sewell WF, Borenstein JT, Chen Z, Fiering J, Handzel O, Holmboe M, Kim ES, Kujawa SG, McKenna MJ, Mescher MM, Murphy B, Swan EEL, Peppi M, Tao S (2009) Development of a microfluidics-based intracochlear drug delivery device. Audiol Neurotol 14(6):411–422. https://doi.org/10.1159/000241898
127. Ayoob AM, Borenstein JT (2015) The role of intracochlear drug delivery devices in the management of inner ear disease. Expert Opin Drug Deliv 12(3):465–479. https://doi.org/10.1517/17425247.2015.974548
128. Gaudana R, Ananthula HK, Parenky A, Mitra AK (2010) Ocular drug delivery. AAPS J 12(3):348–360. https://doi.org/10.1208/s12248-010-9183-3
129. Geroski DH, Edelhauser HF (2000) Drug delivery for posterior segment eye disease. Invest Ophthalmol Vis Sci 41(5):961–964
130. del Amo EM, Urtti A (2008) Current and future ophthalmic drug delivery systems: a shift to the posterior segment. Drug Discov Today 13(3):135–143. https://doi.org/10.1016/j.drudis.2007.11.002
131. McConville J (2016) Special focus issue: ocular and ophthalmic drug delivery systems. Drug Dev Ind Pharm 42(4):513. https://doi.org/10.3109/03639045.2016.1155308
132. Sharma A, Taniguchi J (2017) Review: Emerging strategies for antimicrobial drug delivery to the ocular surface: implications for infectious keratitis. Ocul Surf 15(4):670–679. https://doi.org/10.1016/j.jtos.2017.06.001
133. Sultana Y, Jain R, Aqil M, Ali A (2006) Review of ocular drug delivery. Curr Drug Deliv 3(2):207–217. https://www.eurekaselect.com/article/472. Accessed 10 Jan 2022
134. Gulsen D, Chauhan A (2004) Ophthalmic drug delivery through contact lenses. Invest Ophthalmol Vis Sci 45(7):2342–2347. https://doi.org/10.1167/iovs.03-0959
135. Hui A (2017) Contact lenses for ophthalmic drug delivery. Clin Exp Optom 100(5):494–512. https://doi.org/10.1111/cxo.12592
136. Filipe HP, Paradiso P, Valente ARB, Mata JLG, Saramago B, Henriques JM, Serro AP (2015) Microfluidic in vitro drug release from contact lens materials. Acta Ophthalmol 93(S255). https://doi.org/10.1111/j.1755-3768.2015.0578

137. Li P-Y, Shih J, Lo R, Saati S, Agrawal R, Humayun MS, Tai Y-C, Meng E (2008) An electrochemical intraocular drug delivery device. Sensors Actuators A Phys 143(1):41–48. https://doi.org/10.1016/j.sna.2007.06.034
138. Saati S, Lo R, Li P-Y, Meng E, Varma R, Humayun MS (2009) Mini drug pump for ophthalmic use. Trans Am Ophthalmol Soc 107:60–70
139. Amoozgar B, Wei X, Lee JH, Bloomer M, Zhao Z, Coh P, He F, Luan L, Xie C, Han Y (2017) A novel flexible microfluidic meshwork to reduce fibrosis in glaucoma surgery. PLoS One 12(3):e0172556. https://doi.org/10.1371/journal.pone.0172556
140. Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH (1994) Convection-enhanced delivery of macromolecules in the brain. Proc Natl Acad Sci U S A 91(6): 2076–2080. https://doi.org/10.1073/pnas.91.6.2076
141. Foley CP, Nishimura N, Neeves KB, Schaffer CB, Olbricht WL (2009) Flexible microfluidic devices supported by biodegradable insertion scaffolds for convection-enhanced neural drug delivery. Biomed Microdevices 11(4):915. https://doi.org/10.1007/s10544-009-9308-6
142. Krewson CE, Saltzman WM (1996) Transport and elimination of recombinant human NGF during long-term delivery to the brain. Brain Res 727(1):169–181. https://doi.org/10.1016/0006-8993(96)00378-2
143. Stroh M, Zipfel WR, Williams RM, Webb WW, Saltzman WM (2003) Diffusion of nerve growth factor in rat striatum as determined by multiphoton microscopy. Biophys J 85(1): 581–588. https://doi.org/10.1016/S0006-3495(03)74502-0
144. Szarowski DH, Andersen MD, Retterer S, Spence AJ, Isaacson M, Craighead HG, Turner JN, Shain W (2003) Brain responses to micro-machined silicon devices. Brain Res 983(1):23–35. https://doi.org/10.1016/S0006-8993(03)03023-3
145. Subbaroyan J, Martin DC, Kipke DR (2005) A finite-element model of the mechanical effects of implantable microelectrodes in the cerebral cortex. J Neural Eng 2(4):103–113. https://doi.org/10.1088/1741-2560/2/4/006
146. Uguz I, Proctor CM, Curto VF, Pappa A-M, Donahue MJ, Ferro M, Owens RM, Khodagholy D, Inal S, Malliaras GG (2017) A microfluidic ion pump for in vivo drug delivery. Adv Mater 29(27):1701217. https://doi.org/10.1002/adma.201701217
147. Martins C, Sarmento B (2020) Microfluidic manufacturing of multitargeted PLGA/PEG nanoparticles for delivery of taxane chemotherapeutics. In: Jain KK (ed) Drug delivery systems. Springer, New York, pp 213–224. https://doi.org/10.1007/978-1-4939-9798-5_11
148. Liu D, Zhang H, Mäkilä E, Fan J, Herranz-Blanco B, Wang C-F, Rosa R, Ribeiro AJ, Salonen J, Hirvonen J, Santos HA (2015) Microfluidic assisted one-step fabrication of porous silicon@acetalated dextran nanocomposites for precisely controlled combination chemotherapy. Biomaterials 39:249–259. https://doi.org/10.1016/j.biomaterials.2014.10.079
149. Lababidi N, Sigal V, Koenneke A, Schwarzkopf K, Manz A, Schneider M (2019) Microfluidics as tool to prepare size-tunable PLGA nanoparticles with high curcumin encapsulation for efficient mucus penetration. Beilstein J Nanotechnol 10:2280–2293. https://doi.org/10.3762/bjnano.10.220
150. Tuomela A, Saarinen J, Strachan CJ, Hirvonen J, Peltonen L (2016) Production, applications and in vivo fate of drug nanocrystals. J Drug Deliv Sci Technol 34:21–31. https://doi.org/10.1016/j.jddst.2016.02.006
151. Dong Y-D, Tchung E, Nowell C, Kaga S, Leong N, Mehta D, Kaminskas LM, Boyd BJ (2019) Microfluidic preparation of drug-loaded PEGylated liposomes, and the impact of liposome size on tumour retention and penetration. J Liposome Res 29(1):1–9. https://doi.org/10.1080/08982104.2017.1391285
152. Zhang W, Böttger R, Qin Z, Kulkarni JA, Vogler J, Cullis PR, Li S-D (2019) Phospholipid-free small unilamellar vesicles for drug targeting to cells in the liver. Small 15(43):e1901782. https://doi.org/10.1002/smll.201901782
153. Dovichi NJ, Zhang J (2000) How capillary electrophoresis sequenced the human genome. Angew Chem Int Ed 39(24):4463–4468. https://doi.org/10.1002/1521-3773(20001215)39:24<4463::AID-ANIE4463>3.0.CO;2-8

154. Iv CWS, Reyes CD, López GP (2015) Microfluidic cell sorting: a review of the advances in the separation of cells from debulking to rare cell isolation. Lab Chip 15(5):1230–1249. https://doi.org/10.1039/C4LC01246A
155. Paegel BM, Blazej RG, Mathies RA (2003) Microfluidic devices for DNA sequencing: sample preparation and electrophoretic analysis. Curr Opin Biotechnol 14(1):42–50. https://doi.org/10.1016/S0958-1669(02)00004-6
156. Martin WE, Ge N, Srijanto BR, Furnish E, Collier CP, Trinkle CA, Richards CI (2017) Real-time sensing of single-ligand delivery with nanoaperture-integrated microfluidic devices. ACS Omega 2(7):3858–3867. https://doi.org/10.1021/acsomega.7b00934
157. Bhise NS, Ribas J, Manoharan V, Zhang YS, Polini A, Massa S, Dokmeci MR, Khademhosseini A (2014) Organ-on-a-chip platforms for studying drug delivery systems. J Control Release 190:82–93. https://doi.org/10.1016/j.jconrel.2014.05.004
158. Capretto L, Carugo D, Mazzitelli S, Nastruzzi C, Zhang X (2013) Microfluidic and lab-on-a-chip preparation routes for organic nanoparticles and vesicular systems for nanomedicine applications. Adv Drug Deliv Rev 65(11):1496–1532. https://doi.org/10.1016/j.addr.2013.08.002
159. Zhang YS, Aleman J, Shin SR, Kilic T, Kim D, Shaegh SAM, Massa S, Riahi R, Chae S, Hu N, Avci H, Zhang W, Silvestri A, Nezhad AS, Manbohi A, Ferrari FD, Polini A, Calzone G, Shaikh N et al (2017) Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proc Natl Acad Sci U S A 114(12):E2293–E2302. https://doi.org/10.1073/pnas.1612906114
160. Shallan AI, Priest C (2019) Microfluidic process intensification for synthesis and formulation in the pharmaceutical industry. Chem Eng Process Process Intens 142:107559. https://doi.org/10.1016/j.cep.2019.107559
161. Jensen KF (2017) Flow chemistry—microreaction technology comes of age. AIChE J 63(3):858–869. https://doi.org/10.1002/aic.15642
162. Webb C, Forbes N, Roces CB, Anderluzzi G, Lou G, Abraham S, Ingalls L, Marshall K, Leaver TJ, Watts JA, Aylott JW, Perrie Y (2020) Using microfluidics for scalable manufacturing of nanomedicines from bench to GMP: a case study using protein-loaded liposomes. Int J Pharm 582:119266. https://doi.org/10.1016/j.ijpharm.2020.119266
163. Chang C-H, Paul BK, Remcho VT, Atre S, Hutchison JE (2008) Synthesis and post-processing of nanomaterials using microreaction technology. J Nanopart Res 10(6):965–980. https://doi.org/10.1007/s11051-007-9355-y
164. Amador C, Gavriilidis A, Angeli P (2004) Flow distribution in different microreactor scale-out geometries and the effect of manufacturing tolerances and channel blockage. Chem Eng J 101(1):379–390. https://doi.org/10.1016/j.cej.2003.11.031
165. Ying Y, Chen G, Zhao Y, Li S, Yuan Q (2008) A high throughput methodology for continuous preparation of monodispersed nanocrystals in microfluidic reactors. Chem Eng J 135(3):209–215. https://doi.org/10.1016/j.cej.2007.03.009
166. Li Y-C, Zhang YS, Akpek A, Shin SR, Khademhosseini A (2016) 4D bioprinting: the next-generation technology for biofabrication enabled by stimuli-responsive materials. Biofabrication 9(1):012001. https://doi.org/10.1088/1758-5090/9/1/012001
167. Aziz AUR, Geng C, Fu M, Yu X, Qin K, Liu B (2017) The role of microfluidics for organ on chip simulations. Bioengineering (Basel, Switzerland) 4(2):E39. https://doi.org/10.3390/bioengineering4020039
168. Nguyen N-T, Hejazian M, Ooi CH, Kashaninejad N (2017) Recent advances and future perspectives on microfluidic liquid handling. Micromachines 8(6):186. https://doi.org/10.3390/mi8060186
169. Sackmann EK, Fulton AL, Beebe DJ (2014) The present and future role of microfluidics in biomedical research. Nature 507(7491):181–189. https://doi.org/10.1038/nature13118

Microfluidic-Based Sensors

7

Khairunnisa Amreen and Sanket Goel

Abstract

Off lately, there has been an extensive growth and development in microfluidic technology. This trend is likely to be continued in near future as well. Microfluidics has emerged as a powerful tool for the fabrication of sensors in fields, like diagnostics, clinical analysis, electronics, material science, and drug discovery, owing to their capabilities of using minimal sample volume, accurate and precise flow control, high integration, etc. New innovations, particularly in the interaction of materials with biological systems, hassled to the development of point-of-care testing microdevices. The microscale confinements of the reagent volumes have made these devices quite cost-effective. Furthermore, the emerging concept of organ-on-chips that fabricate microengineered model system of human organs has led to new pathway of disease and infection analysis, drug metabolic and toxicological studies, etc. Likewise, the lab-on-chip concept has made clinical diagnosis rapid and easy to handle. Further, lab-based clinical procedures can now be carried out instantly at home comfort using these microfluidic sensors. These sensors have bridged the gap between the electronics and physiological systems. Presently, the microfluidic-based wearable electronics sensors made up of flexible materials have drawn substantial attention of researchers in diagnosis and healthcare sector. The great impact of microfluidics over various types of sensor systems, material fabrication, etc., has revolutionized the sensor technology and has a tremendous future scope. This chapter focuses on different aspects of microfluidic-based sensors including their fabrication procedures, materials applied, emerging trends in different types of microfluidic sensors, and their

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widespread applications. It also discusses the future outlook and its impact in sensing systems.

Keywords

Microfluidic · Sensor technology · Lab-on-chip · Point-of-care

7.1 Introduction

The emerging trends in sensor technology and the growing demand for point-of-care technology (POCT)-based sensors in various fields, like biomedical, clinical analysis, environment monitoring, defense, aviation, automobile, industries, drugs synthesis, metabolic sensing, marine biology, security, and safety, have enhanced the significance of microfluidic technology. Otherwise termed as lab-on-a-chip sensors, the microfluidic devices are portable and easy to fabricate. Since their first advent in 1990s [1], there has been considerable advances in not just fabrication procedures but also the applications of various types of microfluidic sensors. Small chip-based sensors with specified dimensions ranging from millimeters to centimeters help to perform laboratory-based quantitative, qualitative analysis and detections with ease and handy operating procedures. Microfluidics proffers accurate and controlled flow of analytes in the reaction zones; hence, it gives easy sensing mechanisms and reduces the dependence on bulky instrument. In addition, the flow in these devices can be altered and adjusted as per the desired rate owing to which this approach is suitable to develop miniaturized sensors [2]. The advance research in microfluidic techniques has paved a pathway for developing varied analytical detection platforms for sensing antigens, pathogens, biomarkers, drugs, etc. Unlike the conventional bulk sensing methods, these devices offer added advantages like less requirement of sample and reagent volume, low raw material consumption, safer handling and storage, enhanced accuracy and portability, therefore, cost-effective [3].

Microfluidic-based sensors are systematically designed to control the flow and mixing of analytes and reagents in the microchannels so as to obtain uniform and high sensitivity even with less sample volume. Also, efficient mixing in the microchannels provides good interaction between reagents and analytes, hence, giving more sensitivity. Recently, microfluidic biosensors for biological elements like proteins, biomarkers, DNA, RNA, tumor cells, drugs, and tissue engineering have been fabricated [4]. In addition, there has been extensive growth in concept of disease-on-a-chip and organ-on-a-chip. These devices can biomimic the natural organ with in vitro stimuli [5]. Owing to the fact that there is a widespread need and growth of microfluidic sensors, more approaches for their fabrication are being explored. Apart from paper, glass, and silicon-based sensors, various polymer sheets like polyimide, elastomers like poly(methyl methacrylate) (PMMA), polydimethylsiloxane (PDMS), cyclic olefins, and polycarbonate are also being used as base materials for manufacturing these sensors [6]. Various techniques, like photolithography, soft-lithography, 3D printing, inkjet printing, screen printing,

molding, embossing, laser ablation, and lamination, are adapted widely for microfabrication. The use of these micromechanic techniques for fabrication has made tremendous advancements to develop cost-effective and point-of-care microfluidic-based sensors. The idea of sample in and result out sensing platforms has made this area of research attractive. Recent trends of smart phone-based devices for health management, food industries, and environmental monitoring integrated with microfluidic platform have resulted in reducing testing time and complexities [7]. These sensors have evolved in many aspects ranging from fabrication technologies to materials used, integration of automation, internet of things (IoT), etc. [8]. Microfluidic sensors have established as a standalone discipline with salient features like robustness, user-friendly, high sensitivity, and specificity, instant response [9].

This chapter discusses recent advances in the field of microfluidic-based sensors, their fabrication methods, reported applications, and advances. Although there are significant advances, yet the POCT devices in fields like analytical chemistry, medical diagnosis, biotechnology, agricultural, and environmental monitoring are to be explored more. This chapter also deliberates the limitations and future aspects of microfluidic-based sensors.

7.2 Materials for Microfluidic Sensors

The materials used for fabricating these devices play a key role in achieving the best performance. The materials chosen for making these sensors have to be scrutinized on parameters like total cost of fabrication, ease of modification, integration, and functional output. The advances and progress in this domain depend upon developing novel materials that offer enhanced sensory parameters and reliability. Since the inception of the microfluidic device, silicon and glass, as base, were the pioneer materials. Later on, various kinds of papers, polymers and hydrogels were also adapted [10]. Owing to their feature of high stability and availability, glass and silicon were preferred commonly; however, these materials were a little higher on the costing; hence, their usage for commercialization and large-scale production is limited. Off lately, a cost-effective liquid glass has been designed using silica nanopowder and monomer without clean room and ambient temperature [11]. In addition to this, less expensive polymers, like polymethyl methacrylate (PMMA), polydimethylsiloxane (PDMS), polystyrene and polycarbonate, have also been employed. Among this, PDMS, being less expensive, easy to modify, permeable to gas, has become the most popular material and used widely. Furthermore, PDMS also gives greater biocompatibility and is ideal to be used in flexible electronic device. There are certain limitations like it is challenging to reinforce elements with PDMS and long-term stability of the modified surface. Other polymers like polyimide can be used to fabricate microfluidic sensors by low-cost laser ablation method. This approach is not only scalable but can also be used over various materials.

Furthermore, paper as a base material for microfluidic sensors has also gained significant attention. Due to the porous nature, flexibility, capillary action, wettability, and high surface area, the paper-based microfluidic sensors are potential candidates or bioassay and immunosensing [12]. Electrospun nanofiber-based membranes, because of their large surface area, high porosity, are other potential materials that can be used to design microfluidic sensors. The major advantage of using this is that they provide good sensitivity and lesser signal-to-noise (S/N) ratio. Materials like conductive filaments, for example, poly lactic acid (PLA), polyethylene terephthalate (PET), poly vinyl alcohol (PVA), and acrylonitrile butadiene styrene (ABS) are some of the other materials used for making these devices via 3D-printing approach. While paper and plastic-based microfluidic sensors are more useful on the commercial and industrial front, the silicon, glass, conductive filament, and PDMS-based sensors are used in academia and research laboratories.

7.3 Fabrication Approaches

There are several fabrication approaches depending upon the base material, size, uniformity, and cost for designing the microfluidic-based sensor. Figure 7.1 gives the pictorial representation of the various methods reported so far.

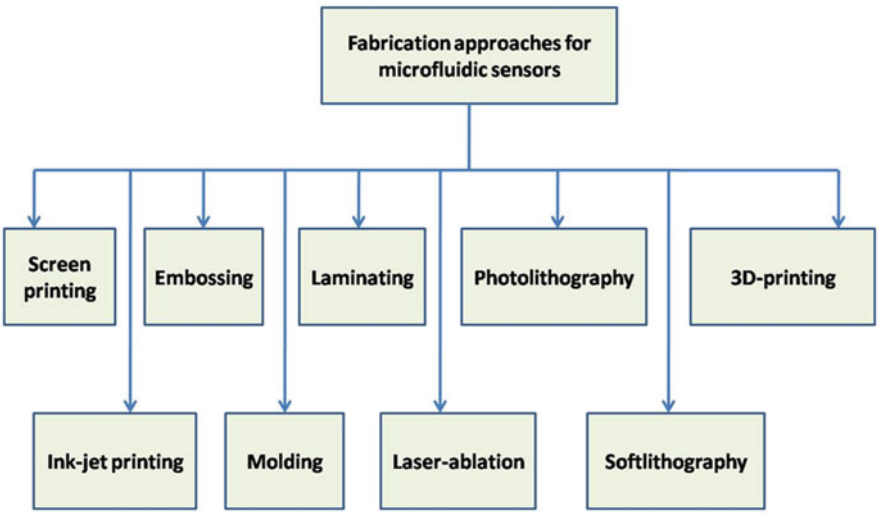


Fig. 7.1 Pictorial representation of the various fabrication approaches for microfluidic sensors used widely

7.3.1 Screen Printing

This method is used for making paper or other flexible substrate-based microfluidic devices. Herein, a wood-based frame is used over which the paper or the flexible substrate is affixed with the help of tape. Specially designed mesh or screen is made from either nylon or silk. These screens act as mask to give desired electrode patterns with specified dimensions. Over this, a conductive ink is poured and dried at temperatures ranging from 60 to 1000 °C to get desired electrode designs. Above this, a PDMS-based microfluidic channel is made and placed to give a uniform flow of analyte [13]. Figure 7.2 gives the schematic representation of the screen-printing procedure.

7.3.2 Inkjet Printing

This is comparatively a modern approach. Herein, a conductive ink of choice with desired viscosity is fed into the cartridge of the inkjet printer. The paper or glass substrate is placed on the base of printing machine and fixed using clamps. The design of the sensor electrodes is fed into the computer in a compatible file format. The distance of the nozzle of cartridge and substrate is adjusted. The printer makes the desired pattern over the substrate. This is then dried and used as sensor. Over this,

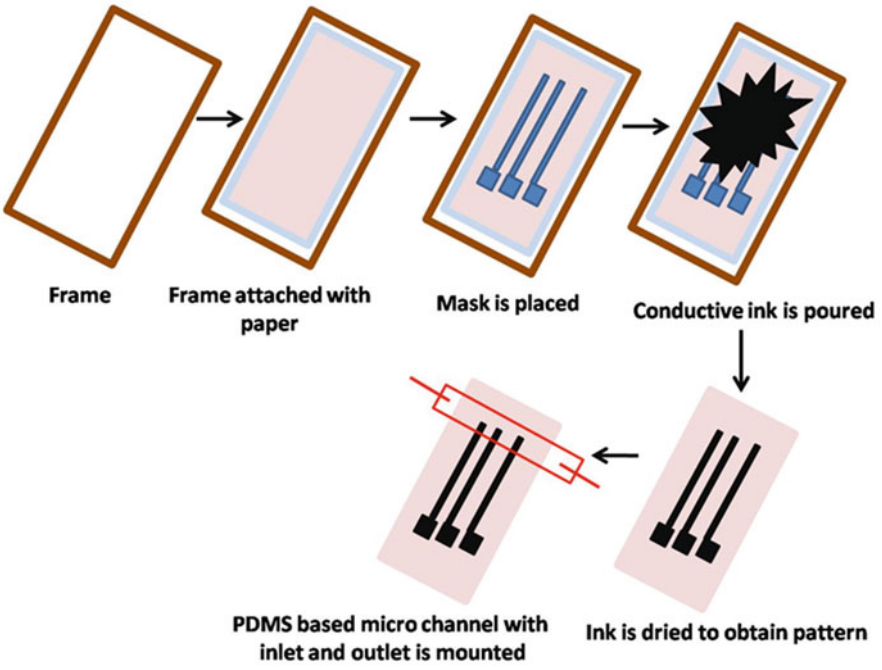


Fig. 7.2 Schematic representation of stepwise procedure of screen-printing procedure

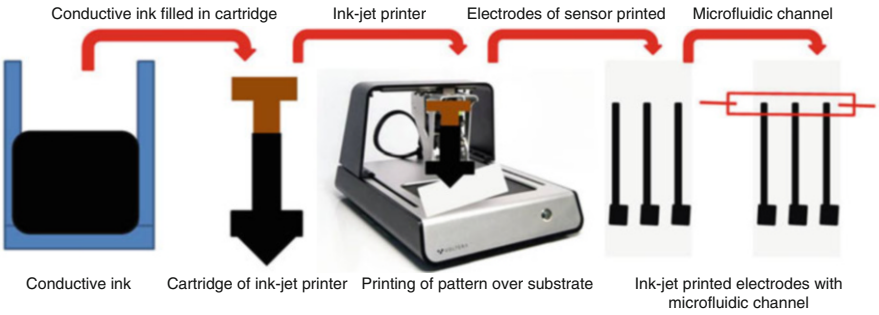


Fig. 7.3 Schematic representation of stepwise procedure of inkjet printing of sensor electrode with microfluidic channel

a PDMS-based microfluidic channel can be affixed [14]. Figure 7.3 is the diagrammatic representation of inkjet printing.

7.3.3 Embossing

Hot embossing is an emerging technique that has capability of reproducing a microscale device over thermoplastics. Herein, a mold of desired pattern of the microfluidic device is made with cavities for microchannels in a microfluidic device. A thermoplastic sheet is placed between this molds whose cavities are at room temperature. Slowly, the temperature is increased and pressure is applied. The thermoplastic melts and fills the cavity of the mold and when pressure is applied the molten thermoplastic takes the shape of the mold. Slowly the temperatures are cooled and the thermoplastic solidifies as the shape of the mold and is ejected [15]. Figure 7.4a is a diagrammatic representation of this method.

7.3.4 Molding

Molding is a technique wherein liquid polymers and thermoplastics are used in molted state. There are two categories in this approach: (1) Replica molding: where a master mold is made with lithography using materials like silicon, over this mold, molten polymer is poured, cured and the solidified. (2) Inject molding: a mold with cavities of microchannels is fabricated using lithographic method and hot molten polymer or thermoplastic is injected in the mold, cavity is filled with. Hot, molten thermoplastic is cooled down to room temperatures and it takes the shape of the microdevice and is ejected from the mold. Figure 7.4b is the schematic representation of the inject molding process [16].

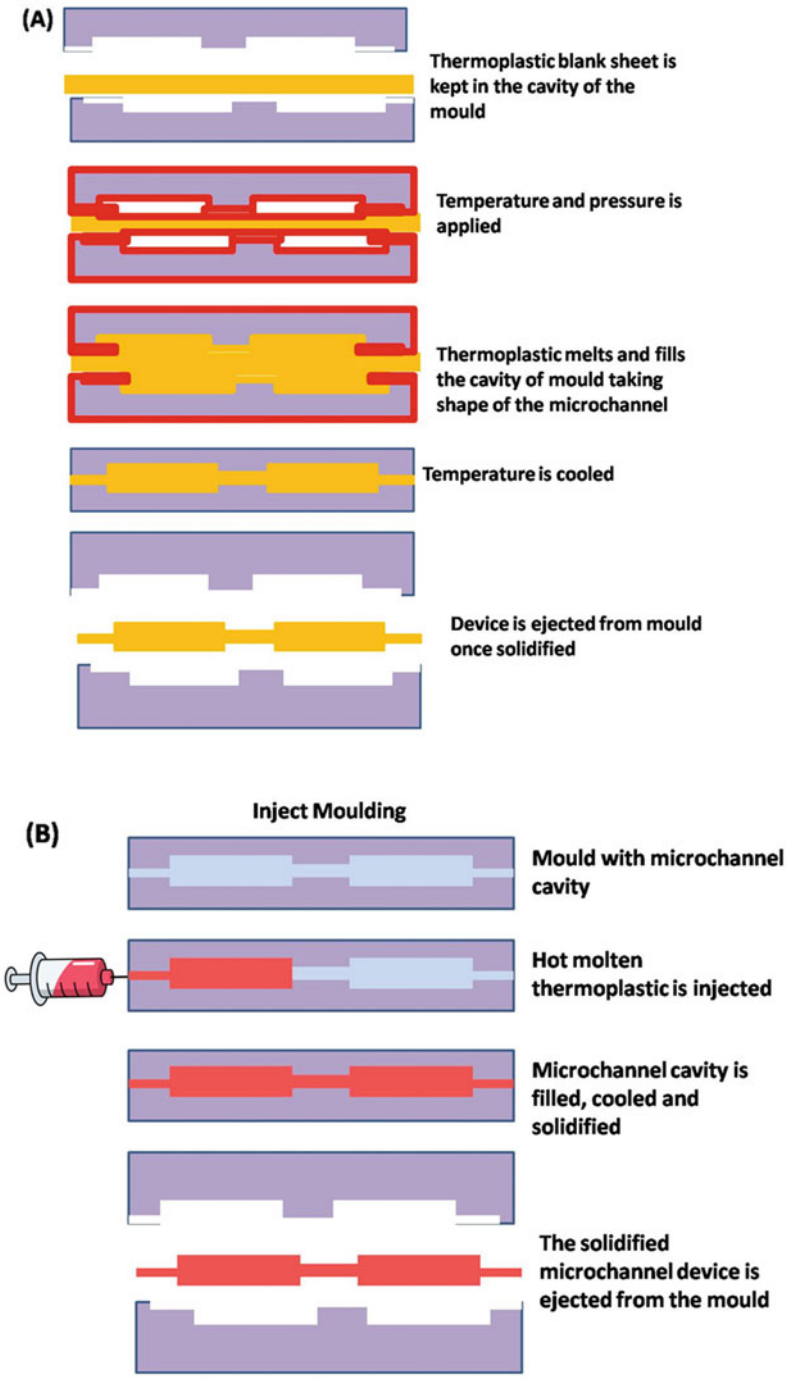


Fig. 7.4 (**a**) Schematic representation of the stepwise procedure of embossing technique. (**b**) Schematic representation of the stepwise procedure of inject molding technique

7.3.5 Laminating

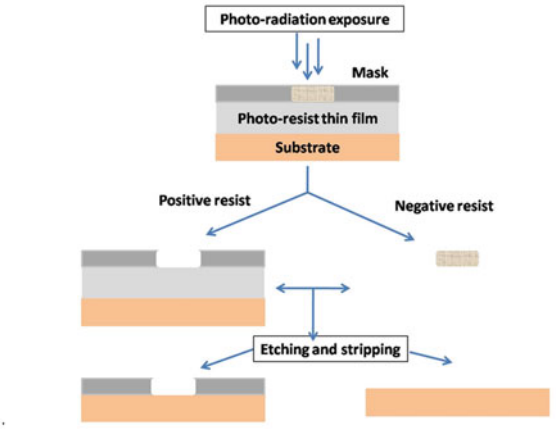
This approach comprises of stacking independently cut layers and bonding them. These types of microfluidic sensors possess three layers: bottom layer, intermediate layer (with microchannel), and a top layer. These layers are bonded to form a firm microdevice integrated with sensor. The number of layers stacked and bonded depends upon the requirement of the device. There are major steps in this procedure: (1) Selection of type of material, (2) cutting the channels on the layer, and (3) bonding the stacked layers. Materials like glass slides, polymers like PMMA, polycarbonate, and PLA adhesive transfer tapes are the materials adapted widely. Knife plotter or laser cut approach (commonly CO_2 laser) is employed for making desired microchannel patterns. Although laser cutting gives an accurate dimension, it is a bit costly and requires skill. In comparison, knife plotter is cost-effective. However, the cutting technique depends on the material used. The use of adhesives, for instance, double-sided tapes, and thermal bonding are the approaches for bonding the stacked layers. In thermal bonding, the temperature of the each layer is raised nearer to the glass transition temperature, then force is applied that bonds the stacked layers into functional firm device [16].

7.3.6 Laser Ablation

Several substrates, like silicon, paper, glass, carbon, plastic, and polymer sheets, can be exposed to laser ablation for designing laser-induced graphene electrodes and laser cut microchannels for sensors. Various types of lasers like CO_2, ultraviolet (UV), pulsed, and diode are employed. Furthermore, different parameters like speed, power of laser, distance, and chemical composition of the substrate also affect the device features and performance.

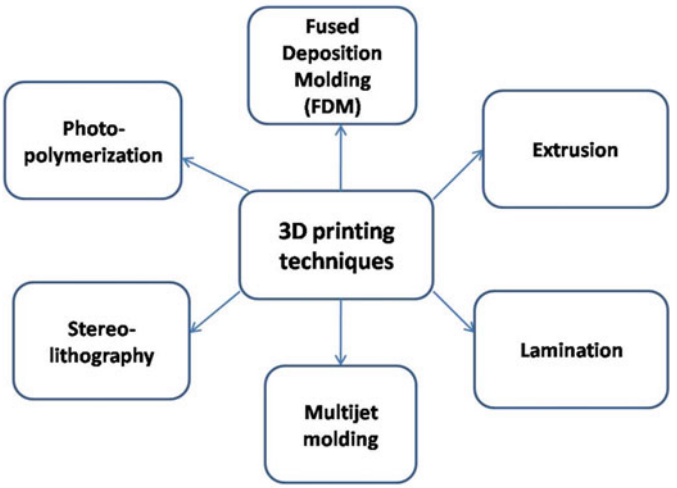
7.3.7 Photolithography

This method makes use of optical means for drawing desired microchannels on the base substrate. Also termed as optical lithography, it is employed to draw channels, patterns, electrodes of microsize for sensing purpose on thin film or bulkier substrates. Earlier, projection of photolithographic techniques can design patterns on smaller substrates like ~250 nm. Nevertheless, the modern photolithographic approach uses soft X-ray, e-beam, focused ion beam, UV beam and can make patters up to 100 nm size as well [16]. Figure 7.5a is the schematic representation of this method.



a: Schematic representation of the photolithography method.

a: Schematic representation of the soft lithographic method of microfluidic device fabrication.



b: Schematic diagram of various 3D printing methods.

Fig. 7.5 (**a**) Schematic representation of the photolithography method. (**b**) Schematic representation of the soft lithographic method of microfluidic device fabrication. (**c**) Schematic diagram of various 3D printing methods

7.3.8 Soft Lithography

This is the most commonly used method of fabrication of microfluidic devices since 1998. Polymers like PDMS are used as a base material here. This is an advance method of self-replicating or replica molding. Herein, liquid materials like polymers (PDMS, polyimide, etc.) will be used to form solid elastomers. PDMS stamp or a master mold is prepared via micromachining. Liquid polymer is mixed with curing agent and poured onto the master with that has device design engraved over it. Elevated temperatures are applied that solidify the liquid. The prepared device is ejected once ready. Figure 7.5b is the schematic representation of this method [17].

7.3.9 3D Printing

This is also termed as additive manufacturing. It is a method of layer-by-layer deposition of desired material. Materials used are the conductive filaments like butadiene styrene (ABS), ploy lactic acid (PLA), polyethylene terephthalate (PET), acrylonitrile wood fiber (Cellulose + PLA), poly vinyl alcohol (PVA). The general procedure includes designing a 3D microfluidic device using a computer aided design (CAD) software. This designed model is converted into a standard triangle language (STL) file that is compatible with the 3D printer software. This software develops a 3D image into a sequential 2D layer giving a G-code file that then the 3D printer can print the device by material deposition. Figure 7.5c gives a summarized diagram of various 3D printing models used [16].

7.4 Microfluidic Sensors

Figure 7.6 gives the general classification of different types of microfluidic sensor. Each type of sensor has a different sensing mechanism. Broadly, microfluidic sensors are of two main types: (1) Microfluidic devices wherein the measurement or sensing of analyte or parameters are measured inside the device, (2) measurement of analytes or parameters externally. Both these mechanisms can be used for preparing physical parameters like pressure, flow, and temperature and biochemical sensors for developing chemical and biosensors. Herein, a few of the remarkable works reported in this chapter for each category are discussed.

7.4.1 Flow Rate Sensors

These microfluidic devices sense the flow rate of different liquids based on their viscosity, time of flow, etc. There are different approaches adapted for these. These sensors are based on principles like pressure, capacitance, and thermal changes as the function of flow rate. For instance, Yu et al. reported a PDMS-based microfluidic

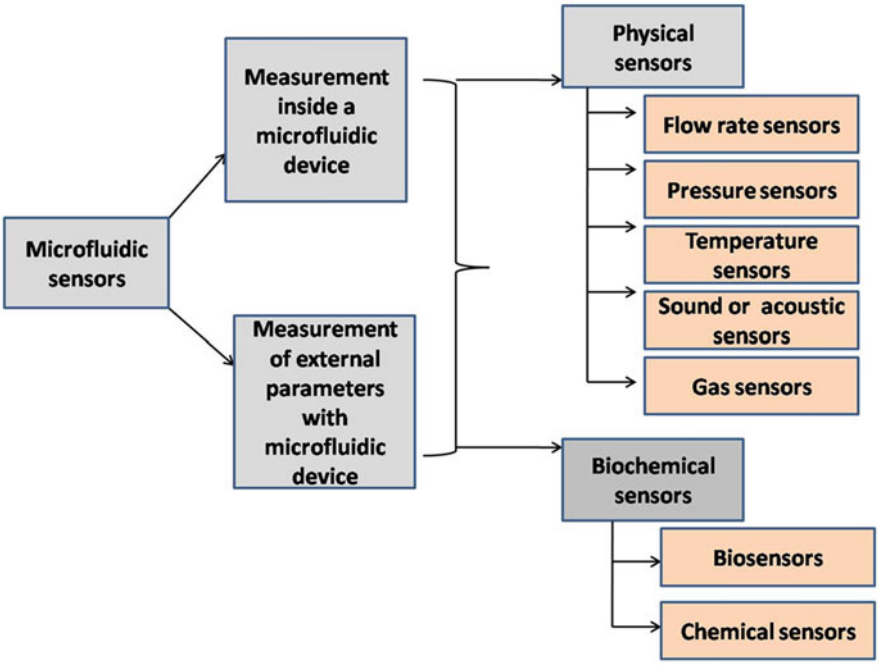


Fig. 7.6 Schematic representation of broad classification of various types microfluidic sensors

sensor for measuring the flow rate based on time of flight. The microdevice has two electrodes placed inside the microchannels. Electrodes are kept at a distant gap allowing the liquid to fill the gap between electrodes. With the help of syringe pump, conductive liquid is made to flow in the microchannels and when it is between electrodes, the resistance will increase. This change in resistance is measured as a function of flow velocity [18]. Dijkstra et al. developed flow sensor based on calorimetric principle. Herein, a microchannel with a temperature cavity is made over substrate. When the fluid is in temperature cavity, it is heated. The temperature changes are measured with the two resistors as a function of flow rate [19]. In another work, Jung et al. developed an integrated microflow sensor. The principle behind this is capacitive pressure of microgas flow. This microfluidic sensor has capacitors that calculate the pressure at the inlet, outlet, and at the microchannel. With the flow in the microchannel, there will be pressure difference that changes capacitance. This is measured as a function of flow [20]. Likewise, Czaplewaki et al. designed a microfluidic flow meter device. Herein, a micromechanical plate of about 100 m^2 was attached with a laser deflection mechanism. Various liquids with different viscosities and flow rates between 2.1 and 41.7 L/min was examined. Syringe pump was used to inject the fluid in the microdevice. Laser deflection was measured as it was directly proportional to the flow rate [21]. Navi et al. developed an underwater microfluidic sensor. It has a dome shaped container with an electrode. The flow of the fluid initiates a drag force to the container and gives displacement

and allows the liquid in the microchannels to flow. The electrode attached converts the displacement observed into the flow rate [22]. Although many approaches are reported but many of these have limitations for practical applications and have a lot of future scope.

7.4.2 Pressure Sensors

One of the significant parameters of microfluidic devices are pressure as the flow of liquid in the microchannels depends upon the pressure. Gauge is the most common method used for measuring pressure but these cannot be applied in microdevices. In these devices, membrane is used in the sensing microchannel, wherein pinching of electrolyte filled microchannels is done. This gives pressure that deforms the elastic membrane. In turn, there is a change in resistance along the microchannel due to membrane deflection [23]. Sekimori et al. fabricated microfluidic chip-based pressure sensor. In this, a 1 mm^3 size pressure sensing element in a glass microchip with diaphragm. This was coated with polycrystalline-SiC. A capacitive sensor is placed. Pressure is measured with change in capacitance in the gap near distortion of the diaphragm [24]. Chen et al. designed a polyimide-carbon nanotube composite-based microfluidic pressure sensor. Multiwalled carbon nanotube (CNT) was used here. The electrical resistivity of this composite material changes with change in pressure and it is used to measure the pressure [25]. Thus, either by measuring the capacitance or resistivity of the microdevices upon applying strain, pressure sensors are developed.

7.4.3 Temperature and Acoustic Sensors

Microscale thermocouples are often used for measuring the temperature. Thin film-based microthermo couples with more spatial resolution are easily fabricated using glass rending excellent performance [26]. Usually, these are embedded into the metals for obtaining firm structure. Herein, wet etching of silicon, electroplating and SU-8 patterning was employed. Nickel metal was used for embedding thermocouples. The changes in the temperature are directly proportional to the thermoelectric output. Their fabricated device was compared with standard thermocouple (K-type) and was found to be same with no error and faster response time nearly 46 ns [26]. Kim et al. designed an integrated microfluidic sensor using a thin film of metal and microchannels for real-time monitoring of parameters like temperature, salinity, and conductivity. The microdevice has a AC voltage supply through power board and DC through 9 V battery a sensing board, mote for wireless connection [27]. In another work, Yang et al. fabricated a microsensor for temperature that was implantable. It was a size of capillary with about 300 μM. The transient temperature of the sensor was analyzed. This device could be used for biomedical diagnosis of hyperthermia [28]. Ye et al. fabricated a novel light-emitting diode

(LED) package with microfluidic temperature sensors that gave precise measurement of temperature [29].

Microfluidics is also being used for developing surface acoustic wave sensing. Earlier, bulk materials based on piezoelectric like lithium and quartz, were used. However, these materials are quite brittle in nature henceforth it becomes difficult to integrate them with electronics for processing the signal. Further, these materials are not useful with multiple modes of waves. Usage of thin films for these sensors makes the integration of electronics with lab-on-a-chip on different substrates including glass, silicon, and polymer. Zhou et al. reported aluminum nitride-based microacoustic devices. Silicon substrate was used here. The prepared device had Rayleigh mode and a higher guided wave mode frequency of wave at 80.3 and 157.3 MHz, respectively [30].

7.4.4 Gas Sensors

Microfluidic gas sensors are used for qualitative and quantitative estimation of specific gases present in the environment in point of care setting. Such sensors are useful to identify gas leakages for integration of a control mechanism for automatic shutdown. The portable microgas sensors have widespread applications in diversified fields like clinical analysis, chemical identification, medical diagnosis, safety, food industry, and defense. Certain significant applications include detection of toxic gases in the environment especially due to emissions from industries, automobiles, etc., safety and security in estimating poisonous gases due to smoke, explosions, fires, etc. Food packaging industries leverage such sensors for detection of gases from rotten food, etc. The literature has some of the benchmarking reports for gas sensing applications. For example, Martini et al. fabricated ammonia gas microfluidic sensor comprising of heater and trioxide tungsten (WO_3) film. This film exhibited selective analysis of ammonia. For this, they subjected the sensor to the test chamber wherein temperature controller maintained the temperature while the concentration of gas was varied. A linear range of 1–100 ppm at 0.2 L/min flow rate was tested. With the decrease in concentration of ammonia, the resistance of the sensor also decreased, that is, resistance was directly proportional to concentration [31].

In another interesting work, Gao et al. developed a microfluidic chlorine sensor via gas–liquid interface using chemiluminescence-based detection by employing luminol alkaline reagent. This liquid solution was trapped in the microchannel due to the applied surface tension. The chlorine gas was introduced into the microchannel that reacts with luminol to form ClO– that emits chemiluminescence. This emitted luminescence was analyzed by a photomultiplier tube in a response time of 30 s. The microdevice exhibited linear range of 0.5–478 ppm with the limit of detection of 0.2 ppm [32]. Yang et al. designed a unique capillary optical fiber-based gas sensor as a ring-shaped waveguide. The inner part of the fiber was coated with organosilicone gel that gave luminescence based on oxygen ratiometry. The oxygen concentration was measured by seeing the luminescence spectra of the

optical fiber [33]. Zhu et al. developed microfluidic sensor for water monitoring by using tin oxide (SnO_2) film-based gas sensor with silicon base microhot plate, which was integrated into a polymer. The device was made via lithography and the SnO_2 was self-deposited. The principle for this sensing was desorption kinetics for different gases in the atmosphere [34]. Ozasa et al. made a unique lab-on-chip device, wherein live microbial cells trapped in the microaquarium helped in gas sensing. The device was made with PDMS. It had a microaquarium with two microfluidic channels. Euglena bacterial cells were present in the microaquarium releasing two gases, that is, air and CO_2 separately. The bacteria cells moved away from CO_2 toward air and this movement was observed with an optical microscopy. This was used to study the concentration gradient of CO_2 [35]. Cooney and Towe demonstrated microfluidic blood gas sensing to monitor CO_2 and O_2 in blood with microdialysis through optical analysis [36]. There is still a large scope for research in this regard as selectivity is an issue in gas sensing. With more precise matrix, materials, enzymes, redox mediators, etc., their sensitivity can be improved.

7.4.5 Microfluidic Biosensors

Biosensors are electrochemical devices that make use of various biological moieties like enzymes, antibodies, cells, microbes, etc., to detect both quantitatively and quantitatively different analytes. These biosensors aim to develop electrical signal that are dependent on the concentration of the analyte. These sensors have three parts: bioreceptor (biological entity), transducer (signal converter), and output reader (analog display). Literature has many biosensors mentioned, wherein bulk volume of reagents and sample is used. However, these are difficult for real-time practical applications. To overcome this, microfluidic biosensor devices are being explored. These microdevices have continuous monitored flow with the help of pumps. These devices have inlets for collecting sample, reaction with reagent, analysis of the reaction, and then signal output readout. There has been immense research for integration of these microfluidic biosensors in day-to-day lives. In this regard, significant reports are there in the literature. The advent of microfluidics has given significant growth in the field of biosensing and there are different types of microfluidic biosensors developed. A few of them that are applicable for real-time application are discussed here.

7.4.5.1 Wearable Microfluidic Biosensors

Emerging trends, like micromachining, microfabrication, and novel material fabrication, have aided the development of wearable microdevices for biosensing that can be worn over skin. The pioneering microfluidic devices were developed for measuring external parameters like pressure, temperature and pH. The new techniques have led to development of wearable microdevices for quantitative and qualitative analysis of various physiological chemicals and biochemicals for monitoring health conditions. For example, Koh et al. developed flexible material based soft, stretchable microfluidic device that can adhere to skin with no chemical and mechanical

means along with wireless integration. The sweat from skin moves through microfluidic network into the reservoir of device. The device has colorimetric reagents for detection of biomarkers in sweat like chloride, glucose, lactate, and hydronium ions. Quantification is achieved by wireless interface that has digital hardware for image capture. The device was tested for real-time analysis on human while straining physical activity like cycling in indoors and outdoors. The device could measure accurately, the pH, lactate, and chloride concentration, sweat rate a sweat loss [37]. Similarly, Cho et al. fabricated capillary action-based soft, microfluidic device that can be mounted on the skin. This device attached to the skin supports collection of sweat sample in the reservoir. Pressure from sweat glands allowed the flow of about 1.8 μL of sweat sample. This device enabled the detection of sodium, potassium, and lactate in sweat [38]. The devices made by the aforementioned groups had three major components. Firstly, an adhesive layer for skin-friendly adhesion, secondly, a PDMS-based microchannel and reservoir containing colorimetric reagents and thirdly a magnetic antenna for wireless transmitting of signals. The four separate reservoirs have serpentine channel for flow of sweat and has reagents for lactate, pH, glucose, and chloride. The outlet on the device top layer avoids the back flow [37, 38]. Sekine et al. made a fluorometric device with smartphone imaging for sweat analysis of biomarkers [39].

7.4.5.2 Paper-Based Microfluidic Biosensors

Paper-based microfluidic devices aim to provide a zero-costing diagnostic tool. In 2010, Martinez et al. developed paper-based diagnostic devices [40]. These devices require hydrophobic barrier for the analyte to flow. The porous nature of the paper due to cellulose allows the fluid to move with capillary action. Various principles like colorimetry, fluorescence, electrochemical, chemiluminescence, and electrochemiluminescence have been used. Among this, colorimetry is widely adapted due to simple and minimal handling and instrumentation. Some of the remarkable paper-based biosensors are discussed here. Gootenberg et al. designed multiplexed RNA and DNA analysis using CRISPR enzymology [41]. This device was applied for real-time detection of ZIKA and Dengue virus RNA with limit of detection as 2aM. This microfluidic platform could also detect mutations in virus from patients' samples. CAS enzyme-based method is expected to have great scope in future. Wang et al. made a pop-up paper-based electroanalytical microdevice for estimation of beta-hydroxybutyrate (BHB) that is a biomarker for diabetes. It was made with a single sheet of paper folded as greeting card into 3D structure. It was made up of a three-electrode system, wherein working electrode was enzyme modified. It was coupled with commercial glucometer and gave detection of glucose and BHB as well [42]. Similar way, Teengam et al. demonstrated a microdevice for DNA, RNA, and colorimetric detection. This was employed for testing DNA of *Mycobacterium tuberculosis*, Middle East respiratory syndrome coronavirus, and human papillomavirus [43]. Ali et al. developed *Escherichia coli* sensor using a composite ink. Herein, a fluorogenic DNA probe was sued. This probe can identify the *E. coli* DNA and gives a fluorescence. A remarkable limit of detection of 100 cells/mL was achieved [44]. Cheng et al. developed paper-based enzyme-linked

immunoassay (ELISA) microdevice. 96-microzone paper-based plate with hydrophobic polymer and hydrophilic paper was made. This device was highly sensitive, robust, and lesser cost [45]. Pollock et al. fabricated a multiplexed device for analysis of biomarkers aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum for liver functioning. The device gave response in 15 min. About 223 real-time samples blood and serum samples were analyzed. The results obtained from the device matched the clinical trials with 90% accuracy henceforth, has potential for real-time analysis [46].

7.4.5.3 Other Substrate-Based Microfluidic Biosensors

Apart from flexible substrates and paper-based microfluidic biosensors, other substrates like PMMA and PDMS are used for fabrication. For example, Sueng et al. developed a PDMS microfluidic chip for detection of Adenosine triphosphate (ATP) in real time. Herein, an aerosol condensation system is employed. The microchannel has ATP bioluminescence transducer as a mediator. The aerosol microbes were settled, bacterial ATP from the cell and concentration is found by bioluminescence. The chip was made through soft lithography with microchannel thickness as 100 μM. There were four inlets of 100 μM, one outlet of 400 μM, mixing channel, detection zone of 6 cm [47]. Hesam et al. designed a PDMS microfluidic device for detection of cancer cells based on the principle that the cancer cells will have lesser mobility than normal cells. Benign and early stage of cancer cells for both breast and ovarian cancer cells. A steady flow rate is maintained and cells are allowed in the microchannels through syringe pump. When one cell passes through the microchannel, another cannot pass. Using an inverted lens microscope, there movement is studied [48]. Using soft-lithography technique, a PDMS device is fabricated for detection of cell flow. The flow causes the changes in conductivity in the microchannel. A printed circuit board is used for realizing electrode plates. Microreservoirs are faced at these plates. The sample with cells is inserted into the microreservoirs, passes through the channel and comes out of other microreservoir. The change in impedance during this process is measured as a function of cell flow [49].

Wisitsoraat et al. developed a PDMS/glass-based microfluidic chip device based on flow injection. It was a three-electrode system device with working electrode as functionalized CNT, silver as a reference, and platinum as a counter. Chemical vapor deposition and sputtering method was used. Cholesterol oxidase was entrapped over CNT in channel. Chronoamperometry was the technique used. The sensor could detect 60 samples. The linear detection range was 50–400 mg/dL. No interference from other co-existing biochemical, like ascorbic acid, glucose, uric acid, and acetaminophen, can be useful for practical application [50]. Nguyen et al. designed interdigitated electrodes with carbon nanotube-polyaniline composite for detection of cholesterol. Potassium ferricyanide is used as a redox mediator. The mediator causes shuttling of electrons. The linear range of cholesterol was 0.02–1.2 mM [51]. Rodrigues et al. developed a glucose biosensor over a PDMS substrate. Herein, gold electrodes with glucose oxidase enzyme were used. Nafion was used to protect the enzyme. The current obtained was directly proportional to the glucose sample

[52]. Li et al. gave detailed review on preparing microfluidic devices with threads. Owing to the capillary action, threads can be employed in microdevices as lesser cost, lesser volume, and simple substrate. These can be employed with paper, PDMS, PMMA and as standalone devices. They have given recent advances in the applications of versatile thread-based microfluidic devices in areas like health monitoring, clinical diagnosis, food industries, and environment monitoring [53]. Thus, advent of microfluidics in the biosensing has significantly enhanced the real-time practical applications.

7.4.6 Microfluidic Chemical Sensors

Microfluidic platforms for various chemical detections are also being realized. For instance, a detailed review on recent advances in microfluidic devices for monitoring environmental pollutants and parameters like pH, heavy metals, nitrite, nitrate, bacteria, phenols, pesticides, and herbicides [54]. Shen et al. fabricated electrochemical microfluidic platform for detection of heavy metals. It was a paper-based device with three-electrode system, wherein pure graphite foil was used as a working electrode along with reference and counter. This device was tested for detection of cadmium and lead. A remarkable detection limit with 1.8 and 1.2 μg/L for lead and cadmium was found respectively. The device was highly reproducible up to ten times [55]. Yin et al. designed a microfluidic sensor for chemical oxygen demand detection. Herein, a silicon-based device was used which consisted of, Ag/AgCl as a reference electrode, platinum as a counter electrode, and PbO_2 modified working electrode. The device was 8 mm × 10 mm dimension. Phthalate derivative detection was performed in the linear range of 4.17–200 mg/L. The limit of detection was 2.05 mg/L. The fabrication of sensor was through microfabrication mass production approach, hence has potential to commercialize in low cost. In further, this sensor has IoT integration facility [56]. Gomez et al. demonstrated detection of Arsenic in water samples using a silicon-based microfluidic device. A three-electrode system comprising of gold nanoparticles gold film electrode as working is employed. The device was fabricated with lithographic method and gave linear detection in the range of 1–150 μg/L with limit of detection as 10 μg/L [57].

Kochli et al. developed microfluidic device integrated with electrochemical sensing for detection of lead and chlorophenols. Lamination was used as the technique of fabrication technique with laser machined polyimide sheet [58]. Wang et al. fabricated a microfluidic device on an acrylic substrate with a microchannel of 1 mm width, 7 mm length and 150 μL volume. The microchannel was coated well with silica gel. Post it dried, *o*-phenylenediamine reagent is coated. The reagent is colorless when NO_2 gas comes in contact with reagent becomes yellow in color [59]. Rérolle et al. demonstrated colorimetric microfluidic pH sensor for real-time application to sea sample. Minimal reagent volume was required [60]. Bowden and Diamond fabricated a microfluidic colorimetric phosphate sensor. Vanadomolybdophosphoric acid complex formation approach was used. The chip was three layered, wherein center layer of Si oxide is kept between glass sheets. The

chip has sample inlets, mixing channels, and optical detection cuvette. UV-LED was used for optical detection and a spectrophotometer [61]. Delany et al. demonstrated electrochemiluminescence on a paper substrate. A screen-printed electrode as a disposable sensor prepared by inkjet printing was used. Ruthenium bipyridyl reaction that gives orange luminescence was employed. Nicotinamide adenine dinucleotide (NADH) and 2-(dibutylamino)-ethanol were the analytes detected. A smartphone-based analysis with digital images was done [62]. Wei et al. designed PMMA-based microfluidic chip for detection of benzoyl peroxide using chemiluminescence. The microchannel had 200 μM width, 100 μM depth, 67 mm length. Luminol was used as the reagent to react with benzoyl peroxide to give chemiluminescence. The real sample of flour was used [63]. Cheng et al. developed an impedance-based microfluidic sensor for highly sensitive detection of perfluorooctanesulfonate [64]. There have been several such reports for microfluidic sensing of various chemicals and biochemicals.

7.5 Future Aspects and Conclusion

Microfluidic technology is an interdisciplinary area of research emerging every day and is yet to be explored to its fullest. Alliance between fluid mechanics and various research areas is needed for future growth. Although significant advances have been done, yet commercialization of microfluidic devices is at the beginning stage. The future and upcoming application of microfluidic-based sensing is the growth in microsystem technology. For instance, implantable microdevices were used for monitoring biological components. Last couple of decades have seen significant growth in microbiodevices. Fields like flexible electronics, wearable electronics, drug analysis, tissue engineering, and biochemical and biomarker detection can revolutionize the applications. The upcoming decades are expected to be exciting for field of microfluidics. Microfluidics in lab-on-chip, organ-on-chip also has greater future prospects. Even though these are closer to real-time living cells, still they face difficulty in fabrication. In the near future, microfluidics is a potential tool for integration with human body for health monitoring and disease diagnosis. In addition to this, microfluidic sensing in environmental monitoring, agriculture science, space research, etc., also has enormous impact on daily life. Some of the significant examples for this are smart contact less sensors, noninvasive detections, tattoo-based sensors, etc. In a couple of decades to come, lab-on-chip and POCT are likely to show more commercialization. This chapter has summarized various advanced microfluidic-based sensing approaches. It also gives details about the fabrication methods and materials used for these types of sensors. Furthermore, types of microfluidic-based sensors and their recent applications have also been discussed.

References

1. Manz A, Graber N, Widmer HM (1990) Miniaturized total chemical analysis systems: a novel concept for chemical sensing. Sensors Actuators B Chem 1:244–288. https://doi.org/10.1016/0925-4005(90)80209-I
2. Jung W, Han J, Choi JW, Ahn CH (2015) Point-of-care testing (POCT) diagnostic systems using microfluidic lab-on-a-chip technologies. Microelectron Eng 132:46–57. https://doi.org/10.1016/j.mee.2014.09.024
3. Gu S, Lu Y, Ding Y, Li L, Song H, Wang J, Wu Q (2014) A droplet-based microfluidic electrochemical sensor using platinum-black microelectrode and its application in high sensitive glucose sensing. Biosens Bioelectron 55:106–112. https://doi.org/10.1016/j.bios.2013.12.002
4. Patil SS, Misra RDK (2018) The significance of macromolecular architecture in governing structure-property relationship for biomaterial applications: an overview. Mater Technol 33:364–386. https://doi.org/10.1080/10667857.2018.1447266
5. Bhatia SN, Ingber DE (2014) Microfluidic organs-on-chips. Nat Biotechnol 32:760–772. https://doi.org/10.1038/nbt.2989
6. Becker H, Gärtner C (2008) Polymer microfabrication technologies for microfluidic systems. Anal Bioanal Chem 390:89–111. https://doi.org/10.1007/s00216-007-1692-2
7. Yager P, Edwards T, Fu E, Helton K, Nelson K, Tam MR, Weigl BH (2006) Microfluidic diagnostic technologies for global public health. Nature 442:412–418. https://doi.org/10.1038/nature05064
8. Mellors JS, Jorabchi K, Smith LM, Ramsey JM (2010) Integrated microfluidic device for automated single cell analysis using electrophoretic separation and electrospray ionization mass spectrometry. Anal Chem 82:967–973. https://doi.org/10.1021/ac902218y
9. Yetisen AK, Akram MS, Lowe CR (2013) Paper-based microfluidic point-of-care diagnostic devices. Lab Chip 13:2210–2251. https://doi.org/10.1039/c3lc50169h
10. Yang X, Wang A, Qiao B, Li JUN (2013) Single-atom catalysts: a new frontier in heterogeneous catalysis. Acc Chem Res 46(8):1740–1748
11. Kotz F, Plewa K, Bauer W, Schneider N, Keller N, Nargang T, Helmer D, Sachsenheimer K, Schäfer M, Worgull M, Greiner C, Richter C, Rapp BE (2016) Liquid glass: a facile soft replication method for structuring glass. Adv Mater 28:4646–4650. https://doi.org/10.1002/adma.201506089
12. Ainla A, Hamedi MM, Güder F, Whitesides GM (2017) Electrical textile valves for paper microfluidics. Adv Mater 29:1–10. https://doi.org/10.1002/adma.201702894
13. Alonso-Lomillo MA, Domínguez-Renedo O, Arcos-Martínez MJ (2010) Screen-printed biosensors in microbiology; a review. Talanta 82:1629–1636. https://doi.org/10.1016/j.talanta.2010.08.033
14. Dong Y, Min X, Kim WS (2018) A 3-D-printed integrated PCB-based electrochemical sensor system. IEEE Sensors J 18:2959–2966. https://doi.org/10.1109/JSEN.2018.2801459
15. Goral VN, Hsieh YC, Petzold ON, Faris RA, Yuen PK (2011) Hot embossing of plastic microfluidic devices using poly(dimethylsiloxane) molds. J Micromech Microeng 21:017002. https://doi.org/10.1088/0960-1317/21/1/017002
16. Gale BK, Jafek AR, Lambert CJ, Goenner BL, Moghimifam H, Nze UC, Kamarapu SK (2018) A review of current methods in microfluidic device fabrication and future commercialization prospects. Inventions 3:60. https://doi.org/10.3390/inventions3030060
17. Xia Y, Whitesides GM (1998) Soft lithography. Angew Chem Int Ed 37:550–575. https://doi.org/10.1002/(sici)1521-3773(19980316)37:5<550::aid-anie550>3.3.co;2-7
18. Yu H, Li D, Roberts RC, Xu K, Tien NC (2013) A micro PDMS flow sensor based on time-of-flight measurement for conductive liquid. Microsyst Technol 19:989–994. https://doi.org/10.1007/s00542-012-1686-7
19. Dijkstra M, De Boer MJ, Berenschot JW, Lammerink TSJ, Wiegerink RJ, Elwenspoek M (2007) Miniaturized flow sensor with planar integrated sensor structures on semicircular surface

channels. In: Proc IEEE international conference on micro electro mechanical systems. IEEE, New York, pp 123–126. https://doi.org/10.1109/memsys.2007.4433031
20. Jang J, Wereley ST (2004) A capacitive micro gas flow sensor based on slip flow. In: Proc IEEE international conference on micro electro mechanical systems. IEEE, New York, pp 540–543. https://doi.org/10.1109/mems.2004.1290641
21. Czaplewski DA, Ilic BR, Zalalutdinov M, Olbricht WL, Zehnder AT, Craighead HG, Michalske TA (2004) A micromechanical flow sensor for microfluidic applications. J Microelectromech Syst 13:576–585. https://doi.org/10.1109/JMEMS.2004.832179
22. Mat Nawi MN, Manaf AA, Arshad MR, Sidek O (2013) Modeling of novel microfluidic based flow sensor inspired from fish canal neuromast for underwater sensing. J Teknol 62:33–38. https://doi.org/10.11113/jt.v62.1316
23. Lichtenberg J, Hierlemann A (2009) Liquid-core, piezoresistive, fully polymer-based pressure sensor. IEEE, New York, pp 10–13
24. Sekimori Y, Yoshida Y, Kitamori T (2004) Pressure sensor for micro chemical system on a chip. Proc IEEE Sensor 1:516–519. https://doi.org/10.1109/icsens.2004.1426214
25. Chen HT, Ko HS, Gau C (2009) The study of polymer pressure sensor by nanocomposites with MWNT and it's characteristics testing. In: 2009 IEEE 3rd international conference on nano/molecular medicine and engineering NANOMED. IEEE, New York, pp 243–248. https://doi.org/10.1109/NANOMED.2009.5559076
26. Datta A, Choi H, Li X (2006) Batch fabrication and characterization of micro-thin-film thermocouples embedded in metal. J Electrochem Soc 153:H89. https://doi.org/10.1149/1.2179218
27. Kim M, Choi W, Lim H, Yang S (2013) Integrated microfluidic-based sensor module for real-time measurement of temperature, conductivity, and salinity to monitor reverse osmosis. Desalination 317:166–174. https://doi.org/10.1016/j.desal.2013.03.007
28. Yang Z, Zhang Y, Itoh T (2012) Development of an implantable micro temperature sensor fabricated on the capillary for biomedical and microfluidic monitoring. Proc IEEE Sens 2012:3–6. https://doi.org/10.1109/ICSENS.2012.6411073
29. Ye H, Van Zeijl H, Sokolovskij R, Gielen AWJ, Zhang GQ (2013) Advanced LED package with temperature sensors and microfluidic cooling. In: Proceedings of the electronic components and technology conference. IEEE, New York, pp 1920–1925. https://doi.org/10.1109/ECTC.2013.6575840
30. Zhou J, Pang HF, Garcia-Gancedo L, Iborra E, Clement M, De Miguel-Ramos M, Jin H, Luo JK, Smith S, Dong SR, Wang DM, Fu YQ (2015) Discrete microfluidics based on aluminum nitride surface acoustic wave devices. Microfluid Nanofluid 18:537–548. https://doi.org/10.1007/s10404-014-1456-1
31. Martini V, Bernardini S, Bendahan M, Aguir K, Perrier P, Graur I (2012) Microfluidic gas sensor with integrated pumping system. Sensors Actuators B Chem 170:45–50. https://doi.org/10.1016/j.snb.2011.01.011
32. Gao ZX, Li HF, Liu J, Lin JM (2008) A simple microfluidic chlorine gas sensor based on gas-liquid chemiluminescence of luminol-chlorine system. Anal Chim Acta 622:143–149. https://doi.org/10.1016/j.aca.2008.05.067
33. Yang X, Zheng Y, Luo S, Liu Y, Yuan L (2013) Microfluidic in-fiber oxygen sensor derivates from a capillary optical fiber with a ring-shaped waveguide. Sensors Actuators B Chem 182:571–575. https://doi.org/10.1016/j.snb.2013.03.076
34. Zhu L, Kimballi C, Sniadeck N, Beamesderfer M, Semancik S, Devoe DL (2003) Integrated microfluidic gas sensors for water monitoring. In: Proceedings of the 7th international conference on miniaturized systems for chemistry and life sciences. (UTAS 2003). IEEE, New York, pp 1231–1234
35. Ozasa K, Lee J, Song S, Hara M, Maeda M (2013) Microfluidic gas sensing with living microbial cells confined in a microaquarium. Key Eng Mater 543:431–434. https://doi.org/10.4028/www.scientific.net/KEM.543.431

36. Cooney CG, Towe BC (2004) Evaluation of microfluidic blood gas sensors that combine microdialysis and optical monitoring. Med Biol Eng Comput 42:720–724. https://doi.org/10.1007/BF02347556
37. Koh A, Kang D, Xue Y, Lee S, Pielak RM, Kim J, Hwang T, Min S, Banks A, Bastien P, Manco MC, Wang L, Ammann KR, Jang KI, Won P, Han S, Ghaffari R, Paik U, Slepian MJ, Balooch G, Huang Y, Rogers JA (2016) A soft, wearable microfluidic device for the capture, storage, and colorimetric sensing of sweat. Sci Transl Med 8:1–14. https://doi.org/10.1126/scitranslmed.aaf2593
38. Choi J, Kang D, Han S, Kim SB, Rogers JA (2017) Thin, soft, skin-mounted microfluidic networks with capillary bursting valves for chrono-sampling of sweat. Adv Healthc Mater 6:1–10. https://doi.org/10.1002/adhm.201601355
39. Sekine Y, Kim SB, Zhang Y, Bandodkar AJ, Xu S, Choi J, Irie M, Ray TR, Kohli P, Kozai N, Sugita T, Wu Y, Lee K, Lee KT, Ghaffari R, Rogers JA (2018) A fluorometric skin-interfaced microfluidic device and smartphone imaging module for: in situ quantitative analysis of sweat chemistry. Lab Chip 18:2178–2186. https://doi.org/10.1039/c8lc00530c
40. Martinez AW, Phillips ST, Whitesides GM, Carrilho E (2010) Diagnostics for the developing world: microfluidic paper-based analytical devices. Anal Chem 82:3–10. https://doi.org/10.1021/ac9013989
41. Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F (2018) Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a and Csm6. Science 360:439–444. https://doi.org/10.1126/science.aaq0179
42. Wang CC, Hennek JW, Ainla A, Kumar AA, Lan WJ, Im J, Smith BS, Zhao M, Whitesides GM (2016) A paper-based pop-up electrochemical device for analysis of beta-hydroxybutyrate. Anal Chem 88:6326–6333. https://doi.org/10.1021/acs.analchem.6b00568
43. Teengam P, Siangproh W, Tuantranont A, Vilaivan T, Chailapakul O, Henry CS (2017) Multiplex paper-based colorimetric DNA sensor using pyrrolidinyl peptide nucleic acid-induced AgNPs aggregation for detecting MERS-CoV, MTB, and HPV oligonucleotides. Anal Chem 89:5428–5435. https://doi.org/10.1021/acs.analchem.7b00255
44. Ali MM, Brown CL, Jahanshahi-Anbuhi S, Kannan B, Li Y, Filipe CDM, Brennan JD (2017) A printed multicomponent paper sensor for bacterial detection. Sci Rep 7:1–10. https://doi.org/10.1038/s41598-017-12549-3
45. Cheng CM, Martinez AW, Gong J, Mace CR, Phillips ST, Carrilho E, Mirka KA, Whitesides GM (2010) Paper-based elisa. Angew Chem Int Ed 49:4771–4774. https://doi.org/10.1002/anie.201001005
46. Pollock NR, Rolland JP, Kumar S, Beattie PD, Jain S, Noubary F, Wong VL, Pohlmann RA, Ryan US, Whitesides GM (2012) A paper-based multiplexed transaminase test for low-cost, point-of-care liver function testing. Sci Transl Med 4:152ra129. https://doi.org/10.1126/scitranslmed.3003981
47. Lee SJ, Park JS, Im HT, Il Jung H (2008) A microfluidic ATP-bioluminescence sensor for the detection of airborne microbes. Sensors Actuators B Chem 132:443–448. https://doi.org/10.1016/j.snb.2007.10.035
48. Babahosseini H, Srinivasaraghavan V, Agah M (2012) Microfluidic chip bio-sensor for detection of cancer cells. Proc IEEE Sensors 2012:3–6. https://doi.org/10.1109/ICSENS.2012.6411375
49. Demori M, Ferrari V, Farisè S, Poesio P, Pedrazzani R, Steimberg N, Boniotti J, Mazzoleni G (2012) Microfluidic sensor for noncontact detection of cell flow in a microchannel. Proc Eng 47:1247–1250. https://doi.org/10.1016/j.proeng.2012.09.379
50. Wisitsoraat A, Sritongkham P, Karuwan C, Phokharatkul D, Maturos T, Tuantranont A (2010) Fast cholesterol detection using flow injection microfluidic device with functionalized carbon nanotubes based electrochemical sensor. Biosens Bioelectron 26:1514–1520. https://doi.org/10.1016/j.bios.2010.07.101

51. Nguyen LH, Nguyen HB, Nguyen NT, Nguyen TD, Tran DL (2012) Portable cholesterol detection with polyaniline-carbon nanotube film based interdigitated electrodes. Adv Nat Sci Nanosci Nanotechnol 3. https://doi.org/10.1088/2043-6262/3/1/015004
52. Pereira Rodrigues N, Sakai Y, Fujii T (2008) Cell-based microfluidic biochip for the electrochemical real-time monitoring of glucose and oxygen. Sensors Actuators B Chem 132:608–613. https://doi.org/10.1016/j.snb.2007.12.025
53. Li X, Tian J, Shen W (2010) Thread as a versatile material for low-cost microfluidic diagnostics. ACS Appl Mater Interfaces 2:1–6. https://doi.org/10.1021/am9006148
54. Kudr J, Zitka O, Klimanek M, Vrba R, Adam V (2017) Microfluidic electrochemical devices for pollution analysis—a review. Sensors Actuators B Chem 246:578–590. https://doi.org/10.1016/j.snb.2017.02.052
55. Shen LL, Zhang GR, Li W, Biesalski M, Etzold BJM (2017) Modifier-free microfluidic electrochemical sensor for heavy-metal detection. ACS Omega 2:4593–4603. https://doi.org/10.1021/acsomega.7b00611
56. Yin J, Zhang Z, Zhang X, Mai Y, Luan A, Xu B, Jin Q (2021) A batch microfabrication of a microfluidic electrochemical sensor for rapid chemical oxygen demand measurement. Analyst 146:1956–1964. https://doi.org/10.1039/d0an02133d
57. Giménez-Gómez P, Baldi A, Ayora C, Fernández-Sánchez C (2019) Automated determination of As(III) in waters with an electrochemical sensor integrated into a modular microfluidic system. ACS Sensors 4:3156–3165. https://doi.org/10.1021/acssensors.9b01286
58. Köchli OR, Schnegg MP, Müller DJ, Surbek DV (2001) Integrated microfluidics/electrochemical sensor system for monitoring of environmental exposures to lead and chlorophenols. Biomed Microdevices 3:331–338. https://doi.org/10.1023/A:1012417000516
59. Wang R, Prabhakar A, Iglesias RA, Xian X, Shan X, Tsow F, Forzani ES, Tao N (2012) A microfluidic-colorimetric sensor for continuous monitoring of reactive environmental chemicals. IEEE Sensors J 12:1529–1535. https://doi.org/10.1109/JSEN.2011.2174218
60. Rérolle VMC, Floquet CFA, Harris AJK, Mowlem MC, Bellerby RRGJ, Achterberg EP (2013) Development of a colorimetric microfluidic pH sensor for autonomous seawater measurements. Anal Chim Acta 786:124–131. https://doi.org/10.1016/j.aca.2013.05.008
61. Bowden M, Diamond D (2003) The determination of phosphorus in a microfluidic manifold demonstrating long-term reagent lifetime and chemical stability utilising a colorimetric method. Sensors Actuators B Chem 90:170–174. https://doi.org/10.1016/S0925-4005(03)00024-8
62. Delaney JL, Hogan CF, Tian J, Shen W (2011) Electrogenerated chemiluminescence detection in paper-based microfluidic sensors. Anal Chem 83:1300–1306. https://doi.org/10.1021/ac102392t
63. Wei L, Zhujun Z, Liu Y (2006) Chemiluminescence microfluidic chip fabricated in PMMA for determination of benzoyl peroxide in flour. Food Chem 95:693–698. https://doi.org/10.1016/j.foodchem.2005.04.005
64. Cheng YH, Barpaga D, Soltis JA, Shutthanandan V, Kargupta R, Han KS, McGrail BP, Motkuri RK, Basuray S, Chatterjee S (2020) Metal-organic framework-based microfluidic impedance sensor platform for ultrasensitive detection of perfluorooctanesulfonate. ACS Appl Mater Interfaces 12:10503–10514. https://doi.org/10.1021/acsami.9b22445

Background and Organ on a Chip

8

Martin Dufva

Abstract

The most widespread cell culture devices such as flasks and titer plate do not fully represent the human physiology. Furthermore, the titer plates have difficulties to interconnect many organs together into an organ system. The motivation for interconnecting organ models is to better predict observations in vitro with the corresponding human reaction. Currently, experimental animals are used to get information on the organism levels. However, there are compelling data showing that there is a poor correlation between results in the animal and the corresponding reaction in humans. The human organ on a chip (HOOC) concept provides a path to replace experimental animal and to better understand relationship and effects between organs. The long-term goal of these HOOCs is to use patient- and donor-derived stem cells and build tiny artificial organs and connect them together to get system-level information. There is a large body of evidence suggesting that these precise, often microfluidics-based, organ models functionally outperform corresponding cultures using traditional methods. The gains of these HOOC are likely caused by excellent mass transfer, ability to position different cell types together and to connect organs in a physiological manner.

Keywords

Human physiology · Organ-on-a-chip · Human-on-a-chip · Microfluidics

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8.1 Introduction

There are some situations when the current in vitro systems and experimental animals fail in function and predictive value. The reason in the current in vitro systems is a batch system with poor abilities to mimic the excellent mass transfers in the body. The most advanced batch systems are likely the Transwell inserts that divide the wells into to compartments and allow for placing cells close to each other to establish physical contact between cells of through or through autocrine and paracrine interactions. The main advantages of these devices are their simplicity and the fact that they are standardized to fit a range of analytical instruments and robots. However, it is more difficult to study the complex interplay between tissues and cells found in vivo as these depend on other organs and cells as well as on the activity of the organism and its surrounding. In the polystyrene-flask, confounding factors such as other cell types and activities are stripped away, and it is therefore sometimes difficult to translate the finding to corresponding activities in vivo. Therefore, researchers have used experimental animals to gain information on the organism level, but this introduces bias as well as variability [1]. It is well known that primates are the best model for humans but costs, long lifetime, ethics and difficulties to make transgenes makes the use of these animal problematic. Instead, rodents are extensively used. However, translating the result from rodent to the clinic is difficult. Only 8% of the animal trials can predict what is going to happen in the clinic with a drug [2]. Researchers and pharma industry are therefore beginning to look at alternatives such as organ on a chip [3, 4] which should be seen as a middle way between the simple devices and an organism. With the induced pluripotent stem cell (iPSC) technology, human on a chip (multiorgan on a chip) enables for instance drug testing on patient specific models in vitro. The current hypothesis is that these have better predictive value than experimental animals. Before we investigate organ-on-a-chip examples, let's take a step back and investigate the current in vitro system in detail to contrast these against the physiology of an organism (mammals).

8.2 Current Cell Culture Devices

From material perspective, plates and flasks are as far away from the in-situ system as possible. Hard polystyrene (PS) typically needs to be coated with cell adhesion protein like fibronectin or other extracellular matrices (ECM). In many cases the cells are secreting their own ECM matrix and in other cases a precoating is needed. Typically, these cultures are 2D where a monolayer of cells layer is cultured. 3D culture is however possible by casing cells in a hydrogel such as collagens and Matrigel. Hence, the bottom of the flask contains a thin layer of hydrogels where cells are embedded in a 3D environment. There are many publications to make 3D artificial tissue slices in plates where some cell types are embedded in the hydrogels and other are sitting on top. The aim is often to improve the function of a specific cell type [5]. The issue with this simple approach is that mass transport is only via the surface of the hydrogels and therefore gradients of factors such as oxygen are to be

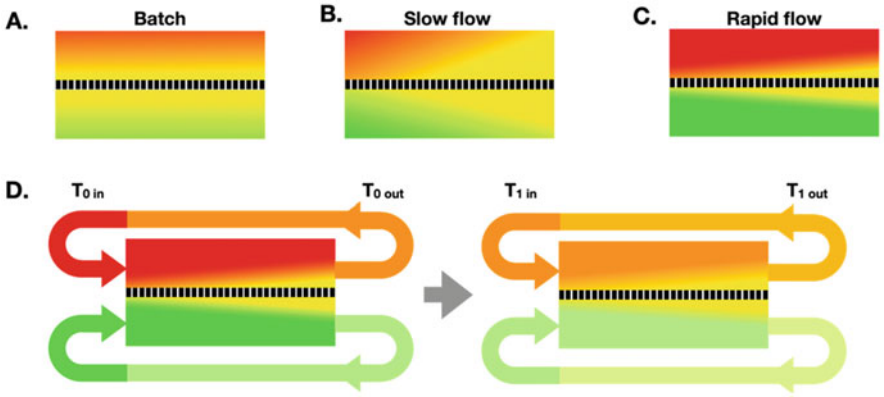

Fig. 8.1 Gradients achieved under different cell culture conditions. (**a**) Situation of cell culture on a filter/membrane. Cells are fed (or releasing factors) from two sides of the filter. Factors are either released from the culture or being used up. Released factors will have highest concentration around the cells and factors that are metabolized are lowest close to the cells. In batch systems, the gradient is rather flat (long distance from the cell layer to the pure medium (red or green)) compared to perfusion systems. (**b**) Perfusion in a sufficiently long channel will also create gradients of factor along the flow direction as well as in the vertical direction and because of that a triangle shape profile is achieved. The gradient is typically sharper than in batch culture. The sharp gradient increases the rate of mass transfer. These gradient profiles will be constant as long as the flow rate and the metabolic rate of the cells are unchanged. (**c**) A higher flow rate will change the gradient profile along the channel to be flatter with clear zones of pure medium on the top (or bottom). In the vertical direction, the gradients will be very sharp ensuring efficient mass transfer. It is possible to completely knock out effects of autocrine factors if the flow rate is sufficiently high. (**d**) Recirculation at two different time points. The resulting gradients will be a mix of **a** (batch) and **b** or **c** (depending on the flow rate). The input concentration of factors will be used up (nutrients) or added to the medium (secreted factors along the channels). The gradient profiles along the channels will change over time because the medium is used up or contains increasing amounts of released factors. In recirculation, the vertical gradients are sharp, but the absolute concentration will decrease over time just like any batch culture

expected inside the gel (Fig. 8.1a). That is not necessarily bad as gradients are also observed in vivo either between two capillaries or along a capillary (Fig. 8.1). Such gel slices are however very slow to react to changes in cues because the mass transfer is only via diffusion.

The PS flasks are batch culture where medium is typically changed every two to three days. At first this seems to be a drawback because the medium composition changes with time. However, some cells depend on autocrine factors and therefore require accumulation of these during culture. It is very difficult to control the secretion of positive acting factors as well as waste in these batch systems because these cultures to a large extent lack homeostasis. In vivo homeostasis is normally achieved through an intricate interaction between different organs and cell types. For instance, the O_2–CO_2 exchange depends on the lung, the red blood cells and the vasculature including the heart in the response to activity. Another example is the blood sugar that is regulated by the beta and alpha cells in the pancreas and the liver

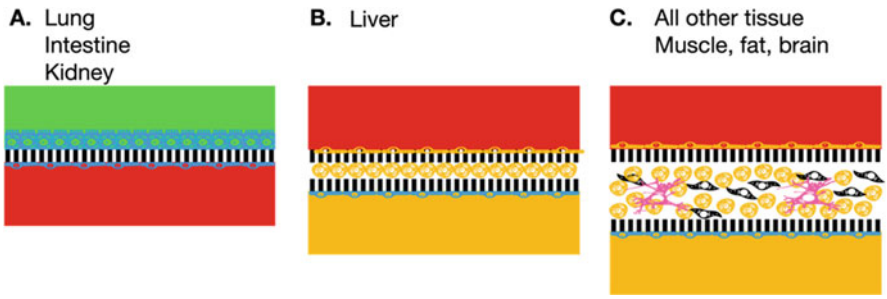

Fig. 8.2 The human body can be reduced to three basic architectures. (**a**) Barriers where there is only a basal membrane between two cell types (typically endothelium and epithelium) are often modelled using a filter like those used in Transwell plates. Short distances are needed to obtain very rapid mass transfer. (**b**) In some organs such as the liver, there is a single layer (or few layers) of cells sandwiched between endothelium and epithelium. Hepatocyte cell in the liver resides in as single layer between the endothelium and the epithelium. The filter used in this example is to help placing the cell correctly according to each other. Normally, the pore of the filter can be considered dead space but modeling the liver, the pores will model the space of Disse. (**c**) All other tissues are surrounded by two channels such as two capillaries or a capillary and lymph vessels. There is always a true 3D architecture of different cells between the two vessels

as a response to feeding and physical activity. There are many of these interaction networks in the body and mostly these are not studied in flasks and wells because it is not easy to physically connect many "organs" together. It also means that batch cultures are in practice uncontrolled in terms of medium composition over time.

An established device for connecting different cells and artificial tissue together is the Transwell inserts as motioned above. These have hard polymeric membrane upon which cells can be cultured on both sides (Fig. 8.1a). The cell layer typically forms a barrier between an upper and a lower fluid compartment. This is the basic unit found in the body: a barrier or tissue between two fluid compartments. For instance, the intestine can be modelled quite well as the upper compartment will correspond to the lumen of the intestine and the bottom is the blood compartment. The epithelium is grown on top of the membrane and endothelium is grown on the opposite side of the membrane facing the lower compartment to get the correct cellular orientation (Figs. 8.1a and 8.2a). There are two types of barriers: one is for fluid/fluid exchange as found in the lung, intestine, and kidney, and the other is a fluid/tissue exchange found in the liver, blood–brain barrier and nearly all other tissues including muscle (Fig. 8.2b, c). A drawback of Transwell inserts is that distances are longer than in the human body. The membrane is a polymeric material with a thickness of 20–30 μm. This seems to be thin, but the intestine–blood and the air/blood barriers have a basal membrane of less than a micrometer. Furthermore, the porosity of artificial membranes is generally low (20–30%).

The distance and the low porosity hinder efficient mass transports. Despite this, it is a convenient and widely used platform to study for instance drug transport over the barriers. The Transwell inserts can also be modified with hydrogels to model the stroma (cell layers between two fluid compartments or tissue function) [6]. It can for

instance contain mesenchymal cells and immune cells. Such a gel layer will however make mass transport even slower from one compartment to another but can recreate the necessary 3D architecture of tissue. A 3D printed three-layer Transwell like system has also been demonstrated where the filter was replaced with a gelatin hydrogel for interconnecting up to three tissue models together [7].

What is lacking in these basic cell culture devices is flow and perfusion to provide shear and to increase mass transfer rates. Inside the human body, we have two closed fluidic networks: the blood vessels and the lymph vessels. In between the cells there is also a layer of liquid called interstitial fluid. Most of the blood is recirculated; however, some is pressed out between the cells becoming interstitial fluids that drains into the lymph system. The latter empties into the vein. About 1/6 of the human body mass is intestinal fluid and 1/12 is blood. Transport of compounds is quick throughout the body. The heart beats about 1 dL/s and with 5 L blood volume and it therefore takes on average about a minute for a compound to reenter the heart after it has been ejected. The reason for these relative rapid transition times is to maximize mass transport in the body by maximizing the steepness of the gradient found between blood (source) and tissue (sink) (compare Fig. 8.1a, c). Another important feature of the body is capillaries that have large surface to volume ratio to ensure rapid mass transfer via diffusion. The distance between two capillaries is maximum 200 μm to ensure that the whole body has good access to O_2, other nutrients and that metabolites are removed. Scaling liquid is difficult where medium liquid (blood/lymph/interstitial fluid) should be about 1/5 of the mass of cells. Typically, a batch culture uses orders of magnitude more medium than the cultured cell mass. The large volume is to avoid changing medium frequently and to avoid the culture to dry out. The drawback is that secreted factors from the cells are diluted but they accumulate rapidly [8] with time because there are no other organs or mechanisms that sequester or degrade these factors.

It is clear form this short review that batch cultures lack fluid dynamics and organ/tissue interaction to model the complexity of an organism. The promise of organ on a chip is to create microphysiological systems that better mimics the complexity of organism but without the whole complexity and poor translation performance of an experimental animals. In this chapter, we will investigate the basic designs of individual organ modules and how to connect then.

8.3 Consideration of Hardware

Before going into specific examples how to solve specific physiological problems on chip, some consideration about the hardware is necessary. This consideration is often HOOC Achilles heel for wider implementation as will be more discussed in the chapter 11 about the future HOOCs.

All microfluidic systems have at least three important parts: the chip, the actuation system that drives the liquid through the chip and the interconnections between the chip and the actuation system. While the chip is tiny on most cases, the actuation system is not. The chip material is not PS but is often made in polydimethylsiloxane

(PDMS) for defining the channels when bonded to glass or PDMS. PDMS chips are produced by casting or injecting molding where the master mold defines the chamber and channels network. The master is produced by microfabrication in clear room processes which allows for structure in the micrometer range which is necessary to model the sizes of structures found in vivo. PDMS is mostly a very good choice for cell culture on chip: it is biocompatible, it has good optical properties, it is transparent to gases including O_2 and it is elastic that allows for incorporation of mechanical features. As PDMS is gas permeable, O_2 level on the chip is nearly never a problem unless of course the intended level is lower than in the atmosphere. It is also possible to vent out excess gases to avoid bubble formations. As it is elastic, it can include on chip valves, pumps, and features that stretch the cell layer [9, 10]. PDMS has however some drawbacks as well: it is not easy to mass produce and it is not inert to some molecules. Small hydrophobic molecules can enter the PDMS (e.g., hormones, fluorochromes, and many pharmaceutical drugs) while proteins interact with the surface of the PDMS. For these reasons, other materials have been investigated like acrylic glass (PMMA) and Topas (cyclic olefin). The reason why PS is not used is that it is difficult to fabricate structures in unless using injection molding and furthermore it is difficult to bond to PS pieces together which typically is required for making a fluidic chip.

The second part of the HOOC system is the actuation to drive the liquid through the system. There are many ways to move liquid on a chip but most often some kind a pump is used. Peristaltic pumps are mimicking the pulsatile flow and can even be incorporated directly on PDMS chips [9]. Peristaltic pumps on the chip are based on three valves that are controlled using an external pressure source (pneumatics). The drawback of these on chip pumps is that the chips become more difficult to produce. An advantage of peristaltic pumps is that they are easy to configure for recirculating the medium which simulates the recirculation in the body. Miniaturized parallel peristaltic pumps with excellent performance have been demonstrated and 3D printing has ensured easy and cost-efficient fabrication [11]. Medium can also be pumped using pressure where the inlet has higher pressure than the outlet. While there are many ways to pump, most have specific limitations, and common to all technologies is that each pump line is costly and complex. This makes parallel systems very expensive.

The third part is the interconnection between the chip and the actuation system. This is often a neglected area but most often provides the biggest challenges in terms of using the device. The reason is that interconnections are the weak link that for instance is a source of bubbles, leakage, and inconsistent pumping. They are also surprisingly expensive and cumbersome to implement on chip. The simplest chip has only one cell culture area, one inlet and one outlet. In biology however, we need that many experiments are performed at the same time to ensure the same conditions for the controls and for the sample as well as having a suitable number of replicates. While dealing with one chip (= one cell culture) is doable, scaling to 16 (16 pump lines and 32 interconnections) or higher is technically very challenging. One of the reasons is that the systems must be compatible with sterile techniques.

The HOOC needs to be "portable." The reason is that they need to be moved from the lateral flow benches (LAF) where the cell loading and medium exchange are taking place to the incubator and microscopes and other instruments. This requirement puts large demand on the design of interconnections and actuation system and it severely limits the total complexity of the system in terms of number of cell culture areas and pump lines.

There are microfluidic solutions that simplify perfusion significantly but comes with limits of versatility. These solutions are based on gravitational actuation where the chip is tilted up and down using a rocker machine. The liquid is therefore moved by gravity from one side to another. The Unichip illustrates that unidirectional flow can be obtained in these systems [12]. The layout of these chips is two wells that are connected via a fluidic channel where the cells are cultured. Operation is simple as the liquid is pipetted into the wells and the systems are gently rocked up and down using a commercial rocker unit (often used to mix blood samples). The drawback of these systems is versatility in pump speed selections. The resistance (length and cross sections) of the channel, the viscosity of the medium and the tilting angle will determine how fast a given liquid moves from one side to the other. To some extent the speed of the flow can be adjusted by the angle of the rocking for a given channel dimension. Mimetas has a titer plate with 40 lanes to model, for example, the intestine where one unit consists of three inlets and three outlets. The middle inlet and outlet are used to fill a cavity with hydrogels. Micropillars spaced in a suitable distance make sure that the hydrogel solution is not going into the adjacent channels. The adjacent channels work as the intestine and blood lumen respectively. Hence, Mimetas chips have the ability to model barriers as well as tissue (Fig. 8.2). It is clear that traditional microfluidics using active pumps, interconnections and chips cannot compete with gravity-based flow for throughput and simplicity.

8.4 Effects of Flow

Flow or perfusion can have large effects on the culture depending on the cell type, the flow velocity, and recirculation. In vivo, it is only the endothelium and the epithelium that are exposed to flow. All other cell types are protected from direct exposure to flow. Therefore, it is likely only the endothelium and the epithelium that can change their molecular composition and functions according to flow because they can sense the shear. Especially the endothelial cells are sensitive to shear stress and cell surface markers like ICAM1 are upregulated under relatively high shear forces. Often however, cells are cultured in perfusion under much slower flow velocity and there are numerous findings that most of the cell types are not affected at all by low shear forces. However, all cells sense their immediate chemical surrounding such as pH, O_2 pressure and secreted factors. Under perfusion, these parameters can be designed to be nearly constant over time (Fig. 8.1b, c). As mentioned above, in batch cultures, a secreted factor like TNF-α increases all the time and reach nM–μM concentration within a day in batch culture [8]. By contrast, under perfusion conditions, the secreted factor is washed out if the medium is not

recircled. Therefore, autocrine or paracrine pathways can be broken by flow as secreted factors are simply washed out (Fig. 8.1b, c). The impact is, for instance, the some cells are not differentiating under flow [8]. Cell types that are unaffected by growth factors or differentiation factors show only minor molecular or phenotypic changes. Cell medium that provides all necessary factors will induce differentiation irrespective of flow [13].

Recirculation (Fig. 8.1d) is popular to achieve as it resembles how the body works. Recirculation requires that the medium is changed regularly as it is used up and waste is accumulating just like in any batch culture. Recirculation also saves medium. The flow without recirculation is typically expensive regarding medium usage. A typical flow rate is 1 μL/min and it seems to be low. However, in 3 days, 4.3 mL medium is used on a cell culture that typically is less than the area of a 96-well plate well that usually uses 200 μL. Recirculation would only require the dead volume of the system which can be considerably lower and in the range of volumes used in batch culture. Both run through (Fig. 8.1b, c) and recirculation (Fig. 8.1d) are usable methods to perfuse cell cultures and the best way depends on the goal of the respective investigation.

8.5 Modelling Physiology on Chip

8.5.1 Fluid/Fluid Interface

A very important aspect of human physiology is the exchange of material between two fluids. This is seen in the alveoli in the lungs, the intestine, and the kidney. Materials such as O_2 or nutrients are moved from the outside (lung or intestine) to the inside (blood) by diffusion. To be efficient, the surface to volume ratio is high and the distance between the outside and the inside is very short. To model this is a challenge as relevant cells need to sit on a thin semipermeable basal membrane (Fig. 8.2a). Membranes can either be hard like those used in Transwell plates or made in PDMS or any other material.

There are several chips that model the intestine. These are very similar to Transwell inserts as it divides a larger chamber into an upper and lower compartment. These chips have therefore four inlets and an outlet to individually control the flow in the respective fluidic channel. The colon cancer cell line (Caco2) is often used to model the epithelium as it, after a 3-week maturation, has similar features as the small intestine. It is a cell line that is also used in the industry for studying drug transport. Curiously there has been several reports that Caco2 cells can start producing mucus when cultured under perfusion suggesting that shear or appropriate feeding can induce a novel function to these cells. Under perfusion and at high seeding densities, the Caco2 layer also matures much faster than in corresponding batch culture [14, 15]. Here is an example where perfusion/shear provides a new function to a cell although the mechanism is unclear.

The next complexity level of modelling the intestine is to create the characteristic villi structure. Villi are important structures that ensure large surface area for

absorbing nutrients from the cells. Its structure also ensures that cells in vivo are matured correctly. A stem cell niche is located in the crypt at the bottom of a villi. These stem cells are gradually differentiating as they move up towards the tip of the villi. Inside the villi however is a network of capillaries as well as the lymph vessels. The capillaries are located very close to the epithelium for rapid mass transfer. It is obvious that the flat membranes and straight channels are not modelling the true 3D environment of intestine villi particularly well but for mechanistic studies the flat membrane is adequate if the permeability and the metabolic activity are similar. Modelling the true 3D structure of the intestine has two challenges. The first one is to create villi and crypt structures to support stem cell division and maturation, just like in vivo. The second challenge deals with making a capillary network inside the villi to ensure adequate mass transfer. Making a villus that is 200 μm in diameter and 400–800 μm high is possible in a range of material. However, the material needs to be a hydrogel for hydrophilic compounds to enter the villi from the outside. Techniques such as casting, sacrificial molding and 3D printing are typically used. On these villi, differentiation of stem cells into a proper multicell type barrier can be achieved [16]. However, the current techniques to 3D print in hydrogels do not allow printing of capillaries or that the blood mimicking channels are close to the surface of the villi. This means that blood vessels are far away (in the range on hundreds of micrometers) from villi surface. As a result, the mass transfer rate per area unit will be relatively low and much lower than in vivo because the hydrogels are much thicker than the basal membrane of the villi. Furthermore, active pumping is required to perfuse these capillaries which are not trivial. Currently, it is possible to get a very good stem cell 3D model [17] for intestine but the mass transfer from the barrier (the epithelium) to the blood model compartment will be very slow due to the long distance from the villi tip to the bottom of the villi unless the villi is perfused. For shorter distance between the epithelium and the endothelium, commercial 2D filters are better as these can be as thin as 10–50 μm which ensured rapid mass transfer.

The lung has much the same issues as the intestine. Also in the lung, the smallest feature where mass transfer happens, has a 3D structure, the alveoli, which is the end point of the larger airways. In this case, the epithelium is very close the endothelium of the capillaries for rapid mass transfer. If we assume that the curvature is not affecting the function cells in the alveoli, it is possible to model the lung using a simple 2D model [18]. The lung however expands and contracts and there are some indications that this movement is necessary for correct activation of the epithelium [10]. The epithelium is not constant over the lung and changes from columnar type of cells in the upper airways to the flat cells in the alveoli. Just like the intestine, also the lung epithelium consists of many types of cells in this case stem cells, goblet cells, ciliated cells, basal cells, and the AT I and AT II cells. Modelling the lung therefore requires recreating different cell compositions found along the airways. The alveoli consists of cell types ATI and ATII sitting on thin basal membrane with endothelial cells in a highly perfused blood vessel network. Higher up, the lung tissue consists of other epithelial cell types sitting on basal membrane that are attached to a layer of smooth muscle cells interspersed with fibroblasts and immune cells. Lung can be modelled using liquid air interface where the epithelium is

directed towards the air and sits of a Transwell insert [18]. The liquid underneath is feeding the cell and models the blood compartment. This air liquid interface has been translated to a microfluidic chip that also provides physical cue to the epithelium [10]. Therefore, the hardware for modelling the lung and the intestine is virtually the same: a membrane simulating the basal membrane that has epithelial cells on one side and endothelial cells on the other side. The challenges are similar in both these organs.

The last example of liquid–liquid interface is the kidney. The kidney's overall function is to clean the blood in a process that artificially is mimicked by dialysis in the clinic. While dialysis cleans the blood by diffusion into a null buffer, the kidney utilized a series or tubes and blood vessels to remove metabolites and drugs while keeping cells, water, sugar, and antibodies in the body. Like the other fluid–fluid interaction tissues, there are two channels in close contact with each other to resorb molecules from the urine into the blood again. Unlike the other interfaces, the kidney also presses liquid from the blood into the Bowmans' capsule and into proximal tubule, loop of Henley and distal tubule where the resorption takes place before being ejected as urine. The filtering between the glomerulus and the Bowman capsule is enhanced by fenestrated endothelial cells which let small molecules and proteins pass but keep the cells and larger molecules in the blood stream. The blood stream runs subsequently along the tubules and resorbs much of the water and smaller molecules. There are many different cell types of epithelial cells in the kidney nephron. These have distinct molecular patterns and have different functions in the resorption process. Reconstituting the whole kidney on one chip is probably not easy because a simple chip layout like two channels divided by a membrane is not sufficient. Such simplistic architectures have however been used to model the proximal tubules [19]. In a normal-sized person, the millions of nephrons are filtering about 110 mL blood/min. Modelling a single nephron would require a flow velocity in the order on 1 μL/min, which is well within the range of a microfluidics chips and pumps.

8.5.2 Modelling 3D Tissue

All other tissues are fed by the capillaries and drained by the veins and lymph systems. Hence, there is barrier layer in form of endo- or epithelium and protects/feeds a 3D structure of other cell types that perform a certain function. The liver for instance is a highly vascularized organ with more than 500 metabolic functions and is a sandwich of endothelium, hepatocytes/Kupfer and stellate cells and epithelium. Mostly the liver is known for its detoxifying activity as well as a key regulator of blood glucose. The liver consists of many vessels and very little tissue in-between (Fig. 8.2b). The smallest functional unit in the liver is the liver acinus. Normally the blood that enters the liver is a mixture of blood from the portal vein (coming from the intestine) and arterial blood and by splitting mechanism ends up in the acinus. Fenestrated endothelial cells are lining the capillaries, and these are very leaky to ensure rapid mass transfer from the blood to the hepatocytes, stellate, and Kupffer

cells. Because the capillary in the acinus is relatively long, and the mass transport and metabolisms are rapid, a gradient from the inlet to the outlet is formed (Fig. 8.1b). Three zones have been identified with distinct pattern of albumin secretion, CYP activity, glucose release, glycolysis, etc. The zones are thought to be defined by the naturally occurring gradients of nutrients and oxygen along the capillary. This is quite easy to model on chip with a sufficiently narrow channel and low flow velocity [20]. Below the endothelial cells are the hepatocytes, stellate, and Kupffer cells that surround the bile duct. To maximize the blood/hepatocyte interactions, there is a space between the endothelium and the hepatocytes (space of Disse). The bile ducts are lined with epithelial cells with very little information about their function. If we neglect the lymph vessels, we can reduce the liver acinus to two channels that sandwich a monolayer of hepatocytes with some interspersed Stellate and Kupffer cells. It can therefore be modelled using two membranes that are stacked on top of each other with a narrow space in-between for hepatocytes, stellate, and Kupffer cells. As opposed to the barriers as discussed above, this tissue is designed for rapid interaction between the blood and the metabolizing cells. While the barriers provide a very tight control what is going in and out of the fluid vessels, the liver's metabolism controls how much of toxic compounds are removed when blood is passing the liver. In organ on a chip system, the intricate network of bile capillaries, bile ducts, and lymph is often reduced to a perfused straight channel, possibly with two channels sandwiching a membrane architecture or a porous structure that directly interacts with the medium (Fig. 8.2a, b).

Many other organs have true 3D structure between capillaries and lymph. This includes muscle, fat, skin, and vascular wall that can be modelled with two membranes sandwiching a void with a hydrogel (Fig. 8.2c).

8.6 Major Blood Vessel Routs in the Body and Mapping to Devices

The human body has three major fluid blood loops: heart to lung, heart to brain and heart to the rest of the body. The lung loop ensures gas exchange, and the brain loops ensure that the brain is well fed with nutrients and oxygen. The rest of the body is mainly connected in parallel where the artery splits into minor vessels (Fig. 8.3). Hence, the organs are all fed with the same composition of the blood with regard to nutrients and oxygen. The different organs get different fraction of the volume of blood pumped from the heart.

During rest, the muscle and kidney get about 20% of the cardiac output while the intestine, brain and the liver get around 10%. All other organs get less than 10% of the cardiac output. The size of the organs, function, and activity determine the relative fraction of the blood flow to the respective organ. For instance, muscle increases its fraction of blood flow during exercise on the expense on all other organs. An exception to the parallel connections is the portal vein which goes from the intestine to the liver. The reason for this route is that the blood that has absorbing molecules from the intestine is "cleaned" of unwanted substances before the blood is

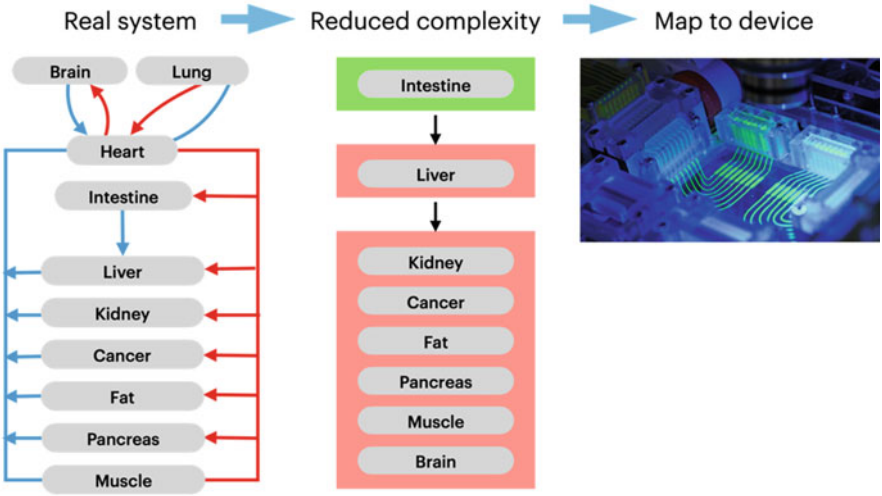

Fig. 8.3 The fluidic network existing in the body is often reduced to a simple on chip. The reason is that it is physically difficult to build the complex fluidic regulation and that the systems are often very difficult to use. An organ can thus be a simple flow through device or recirculation can be employed. As not all organs are usually on a HOOC, some of the fluidic networks will be less important which simplify the fluidics

spread of the body. In many HOOC, the fluidic network is simplified significantly (Fig. 8.3) and reduced to an input, an organ model and an output (single OOC) that type is connected with each other in series or in parallel.

References

1. Richter SH, Garner JP, Würbel H (2009) Environmental standardization: cure or cause of poor reproducibility in animal experiments? Nat Methods 6:257–261
2. Mak IWY, Evaniew N, Ghert M (2014) Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res 6(2):114–118
3. Ingber DE (2020) Is it time for reviewer 3 to request human organ chip experiments instead of animal validation studies? Adv Sci 7:1–15
4. Sung JH, Wang YI, Narasimhan Sriram N, Jackson M, Long C, Hickman JJ, Shuler ML (2019) Recent advances in body-on-a-chip systems. Anal Chem 91:330–351
5. Caliari SR, Burdick JA (2016) A practical guide to hydrogels for cell culture. Nat Methods 13: 405–414
6. Chu HY, Chen YJ, Hsu CJ, Liu YW, Chiou JF, Lu LS, Tseng FG (2020) Physical cues in the microenvironment regulate stemness-dependent homing of breast cancer cells. Cancers 12:1–21
7. Leth Jepsen M, Willumsen A, Mazzoni C, Boisen A, Hagner Nielsen L, Dufva M (2020) 3D printed stackable titer plate inserts supporting three interconnected tissue models for drug transport studies. Adv Biosyst. https://doi.org/10.1002/adbi.201900289
8. Hemmingsen M, Vedel S, Skafte-Pedersen P, Sabourin D, Collas P, Bruus H, Dufva M (2013) The role of paracrine and autocrine signaling in the early phase of adipogenic differentiation of adipose-derived stem cells. PLoS One. https://doi.org/10.1371/journal.pone.0063638

9. Gómez-Sjöberg R, Leyrat AA, Pirone DM, Chen CS, Quake SR (2007) Versatile, fully automated, microfluidic cell culture system. Anal Chem 79:8557–8563
10. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE (2010) Reconstituting organ-level lung functions on a chip. Science 328:1662–1668
11. Jönsson A, Toppi A, Dufva M (2020) The FAST Pump, a low-cost, easy to fabricate, SLA-3D-printed peristaltic pump for multi-channel systems in any lab. HardwareX 8:1–19
12. Wang YI, Shuler ML (2018) UniChip enables long-term recirculating unidirectional perfusion with gravity-driven flow for microphysiological systems. Lab Chip. https://doi.org/10.1039/c8lc00394g
13. Starokozhko V, Hemmingsen M, Larsen L et al (2017) Differentiation of human induced pluripotent stem cell under flow conditions to mature hepatocytes for liver tissue engineering. J Tissue Eng Regen Med 12:1273–1284
14. Tan H-YH-Y, Trier S, Rahbek ULUL, Dufva M, Kutter JPJP, Andresen TLTL (2018) A multi-chamber microfluidic intestinal barrier model using Caco-2 cells for drug transport studies. PLoS One 13:e0197101
15. Kim HJ, Ingber DE (2013) Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation. Integr Biol 5:1130
16. Nikolaev M, Mitrofanova O, Broguiere N et al (2020) Homeostatic mini-intestines through scaffold-guided organoid morphogenesis. Nature 585:574–578
17. Kasendra M, Tovaglieri A, Sontheimer-Phelps A et al (2018) Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids. Sci Rep. https://doi.org/10.1038/s41598-018-21201-7
18. Bluhmki T, Bitzer S, Gindele JA et al (2020) Development of a miniaturized 96-Transwell air–liquid interface human small airway epithelial model. Sci Rep. https://doi.org/10.1038/s41598-020-69948-2
19. Sochol RD, Gupta NR, Bonventre JV (2016) A role for 3D printing in kidney-on-a-chip platforms. Curr Transplant Rep 3:82–92
20. Moradi E, Jalili-Firoozinezhad S, Solati-Hashjin M (2020) Microfluidic organ-on-a-chip models of human liver tissue. Acta Biomater 116:67–83

Culture and Co-culture of Cells for Multi-organ on a Chip

9

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Abstract

The pre-clinical trials in the drug discovery timeline involve an immense amount of time and money to elucidate the drug responses precisely. Cell culture techniques have been utilized for decades to understand the in vivo responses and aided in many research areas, including tissue engineering, biomedical engineering and the pharmaceutical industry. Microfluidic cell culture provides a novel cell culture technique that allows the control of the local environment using microscale dimensions and mimic the human circulatory system. Organ-on-a-chip technology provides a promising alternative for animal models and can better recapitulate the physiological environment with the help of human-derived cell sources. The 3D cell culture provides more physiologically relevant responses than the 2D. The cells in a 3D cell culture are surrounded by the extracellular matrix (ECM) that could enhance the growth and physiological responses. Many significant advances in organ-on-a-chip development integrated with microfluidics have opened the gateway for integrated cell culture techniques and multi-organ cell cultures. The co-culture of cells with the support of various membranes and scaffolds also provides a futuristic application in drug development and disease modelling. The remarkable properties of hydrogels provide more reliable cell culture support and better experimental results. This chapter focuses on the importance of co-culture systems and multi-organ on a chip for various biomedical applications.

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3D cell culture · Co-culture · Multi-organ on a chip · 3D scaffolds

9.1 Introduction

The research and development in the drug development processes have encountered several barriers in selecting a precise pre-clinical model that could perfectly elucidate the physiological responses. The search for an alternative for animal testing has travelled a long way since decades. Cell culture techniques are the most reliable and widely accepted techniques that mimic the in vivo conditions. The cell culture techniques, since its beginning, aided in the research and development of various fields of biology, including cell biology, pharmacology, biomedical engineering and other interdisciplinary areas. The primary aim of cell cultures is to elucidate the cellular interactions that mediate tissue engineering, disease mechanisms, mechanism of action of drugs, protein synthesis and other cellular functions. Cell cultures also play an essential part in drug development, cancer research and toxicology studies. The selection of appropriate cell culture techniques can be the first step in the success of these research areas. The cell behaviour greatly depends on the type of cell culture involved [1].

Many methods and assays involving the traditional cell culture have become an indispensable part of the research worldwide in biology and its related fields. Even though these in vitro cell culture assays have been utilized in many remarkable discoveries, the major limitation of the technique is that it cannot mimic the dynamic conditions inside the biological systems. The 2D cell cultures might have simultaneously utilized the co-culture of two organs. The growth and development of the cells in a static environment are entirely different from a very dynamic and highly complex in vivo environment. The 2D cell cultures cannot entirely mimic the complex functions occurring in a 3D environment. The plastic surfaces used in 2D cultures cannot ultimately provide human physiological behaviour.

The 3D cell environment involves the interaction of cells with their surrounding cells and the extracellular matrix (ECM). Other systemic factors are also involved in the interactions of cells and host a fully functional biological system. However, other than the proliferating nature of the 2D in vitro cultures, they are incapable of mimicking an organ function. Hence, it is highly essential to develop biologically relevant models and thereby enhance the predictability of assays. The development of physiologically relevant cell culture models witnessed an evolution since 3D cell culture systems were introduced. The biomedical and pharmacological assay systems that utilized the traditional 2D cell cultures endured a paradigm shift with 3D cell culture techniques (Fig. 9.1). Understanding the importance of the extracellular matrix and its contribution to the cellular environment is essential to expand the limitations of in vitro modelling [2].

The co-culture of cells has been adopted for a long time to better understand the biological systems as the cellular environment itself is an interconnection of various

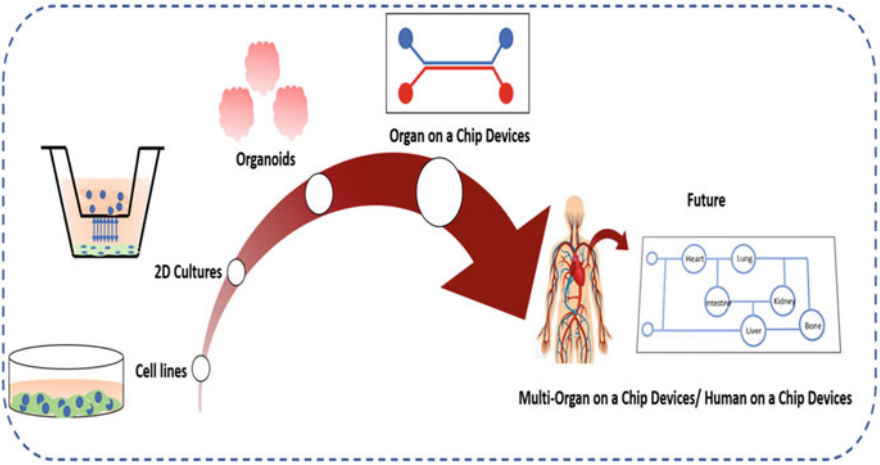

Fig. 9.1 Schematic diagram showing the cell culture from conventional to multi-organ-on-chip systems

cell types, vasculature, cellular pathways and the action of several metabolites. Co-culture refers to the culture of two or more cell types with some connecting factor between them. The systems help to study the various interactions between cell populations and establish synthetic interactions between cell populations. Understanding cell–cell interactions is highly essential to predict any in vivo responses that arise from applying any extracellular cues, including chemicals and drugs. The establishment of a synthetic interaction between cell cultures is essential to produce a model for assessing the physiological function, including drug discovery applications. Co-culturing systems have the advantage of experimental control of the various factors and can be modified accordingly. Hence, many future applications involved in biological experiments are intended to be incorporating co-culture systems [3].

Co-culture systems also play an essential role in understanding the tumour environment and enhancing the cell culture studies involving cancer studies. The tumour microenvironment is highly complex and involves human-specific biophysical and biochemical factors that are difficult to capture in monolayer flask cultures. Hence, the tumour-on-a-chip devices incorporating the various tumour tissues can be a successful model that could elucidate the specific functions. The tumour-on-a-chip platforms with the co-culture of various tissues involved can be pivotal in cancer biology studies [4]. The 3D cell culture also exhibits the limitations of the incapability of exactly mimicking the microenvironment niche. The recent breakthrough in advancing 3D cell cultures is the microfluidic culture systems. The ability of microfluidic cell cultures to capture the dynamics of the microenvironment, including the nutrient supply, oxygen transfers and waste removal, allows them to mimic the circulatory system. In this chapter, the different culture and co-culture of cells in the multi-organ on a chip is defined in terms of the various cell sources used, the

techniques used to culture cells and the development made in the area so far with the multi-organ-on-a-chip cell culture approach.

9.2 Why Do We Need Multi-organ Cell Culture?

The purpose of developing organ-on-a-chip (OOC) platforms is to utilize in vitro culture systems to mimic the in vivo microenvironment and capture the physiological responses closely. The OOC systems or the micro-physiological systems are employed to recapitulate the microscale functions, including the cellular interactions, cell–matrix interactions, transport of nutrients and waste materials, and the mechanical factors that make up the cell niche [5]. The OOC can be classified into two main categories: Firstly, the chip that integrates a specific organ or multiple cells of a single organ to mimic the organ functions. Over the previous decade, there has been an overwhelming development in OOC technology that established many on-chip models of organs, including heart, kidney, lung, liver, skin, eye and muscle. The recent development in single OOC devices is described below.

A novel heart-on-a-chip microdevice was developed by culturing cardiac microtissues derived from human iPSCs. The device could target the kinetics of cardiac microtissue pulsation and quantify some physiological parameters like the fluid output, pressure and force. The device also validated the performance of the heart-on-a-chip microdevice for pharmacological investigations using human iPSCs [6]. A microfluidic platform was fabricated for engineering cardiac microtissues using direct laser writing (DLW) lithography and soft lithography. The device contains four separate parts containing a cardiac microtissue and is attached to an integrated strain actuator and a force sensor. The system also employs cardiac microtissues derived from human-induced pluripotent stem cells (hiPSCs). The device was intended to study the effects of drug screening on cardiac microtissues [7].

The understanding of renal physiology requires efficient in vitro kidney models. Primary cells derived from adult kidney tissue are utilized to develop kidney tubuloids. These cells are embedded in a perfused microfluidic device and are demonstrated as a powerful tool to mimic kidney functions [8]. Another study focused on developing a kidney spheroid on a chip to analyse the nephrotoxicity induced by drugs. The human spheroids on a chip with vascularization and sensors embedded to evaluate the drug-treated cellular responses. The results indicated that the exposure of cyclosporine and cisplatin disrupted the polarity of proximal tubules, and the glucose accumulated toxicity [9].

The need for a physiologically relevant lung model has led to various lung-on-a-chip models. A very recent development is the human alveolar lung-on-a-chip model embedded using gelatin methacryloyl (GelMA). The device was able to capture the cyclic breathing motions. This study developed a unique technique to recapitulate the alveoli in vitro and could be the beginning of the emergence of physiologically relevant distal lung models [10]. Using nanofibrous membranes, 3D printing

technology was utilized to fabricate a breathable lung on a chip. The device was used as a model of ventilator-induced lung injury. Lung alveolar epithelial cells were used to validate the study. The device was introduced as a low-cost and highly adaptable lung-on-a-chip device that has intended applications in predicting the drug responses [11].

The complicated physiology of organs such as the liver makes it difficult the development of reliable liver-on-a-chip models. The construction of a liver model can accentuate the research and development of new drugs and accelerate the drug screening process. However, many studies have come up with the successful fabrication of liver-on-a-chip devices. Hepatocellular carcinoma HepG2 and human hepatic stellate cell LX-2 cells were cultured in a hydrogel-based chip to mimic the structural features of the hepatic lobule. The spheroids formed by the hepatic cells exhibited increased viability and liver-specific functions in comparison to the static cultures. The liver-specific functions showed increased expressions in the presence of endothelial cells. The liver-on-a-chip model proposed in this study exhibits a novel approach for fabricating 3D in vitro liver models. These findings point to drug toxicity screening and artificial bio-liver [12]. A hepatic sinusoid on a chip was developed by 3D printing that enables a drug screening model and toxicity evaluation on a chip. Endothelial cells and hepatocytes were seeded on the device using a 3D printer. The expression of liver biomarkers was compared with monolayer 2D cultures, and the results indicated that the device was able to recapitulate hepatic function much more efficiently than the 2D models [13].

The second type of organ on a chip integrates multiple organ-specific tissues to form an in vivo model. Since the pioneer attempt to develop multiple organ devices using interconnected cell culture compartments called the cell culture analogue, the multi-organ cell culture has witnessed transformations [14]. The integration of multi-organs interconnects the missing link between the animal models and the in vitro cell assay systems. The multi-organ on a chip technology establishes the concept of circulation via the media flow, thereby deciphering the utilization, synthesis and transport of metabolites when a chemical is being added to the media. This, in turn, helps in understanding the absorption, distribution, metabolism, excretion and toxicity profiling of a specific drug in the dynamic environment without the help of animal models. Hence, multi-organ on a chip can be reliable models for predicting the pharmacokinetics and pharmacodynamic of drug molecules and a better alternative for animal testing.

9.3 Sources of Cells in Multi-organ on a Chip

The growth of cells inside the multi-organ on a chip is the primary factor determining the device model's success. In theory, the multi-organ on a chip can accommodate all types of cells ranging from normal cells, cancerous cells, primary cells, embryonic stem cells (ESCs), induced pluripotent stem cells (iPSC) and adult stem cells (ASCs). However, the practical difficulty in using ASCs and ESCs makes way for primary cells and commercially available cell lines to be used more prominently.

The selection of the cell types for a specific multi-organ on a chip will depend upon the type of function expected from the device. Also, the cell culture method used in the device determines the type of cells to be used. The various cell sources used for co-culture in multi-organ on a chip are summarized in Table 9.1.

9.3.1 Cell Lines

The commercially available cell lines have been used to develop organ on a chip for almost all organs. Immortalized cell lines are the most common cell sources used to develop OOC devices. They are easier to handle compared to other cell types. Their indefinite proliferation under specified conditions makes them cost-effective compared to other cell types. Combining these cell lines is used in multi-organ on a chip to study their interactions [27]. The use of cell lines in multi-organ on a chip provides reproducible results as they are a homogenous type of populations and source of mature cells. However, these cells differ in terms of genetic diversity despite being simple to handle and culture.

9.3.2 Primary Cells

The limitations of cell lines are that they lack specificity and limit their use in disease modelling. The use of cell lines in toxicity testing is widespread, but they are limited to assessing known mechanisms of toxicity. Certain cell lines often produce overexpression of proteins that play a significant role in toxicity related pathways [28].

Primary cells are cells obtained directly from human tissue. They are regarded as a better option for predicting the human physiological responses than the cells derived from the tumour. Many studies were carried out to compare the results of primary cells and tumour-derived cell lines [29]. However, both primary cells and cell lines have been interconnected to produce multi-OOC devices for various biomedical applications.

A co-culture of bronchial MucilAir culture and HepaRG cells was done in a multi-organ chip to assess the potential toxicity of inhaled substances under conditions that permit organ cross-talk. HepaRG cells and HHSteCs were used to form liver spheroids and were co-cultured with MucilAir culture. This model may be intended to determine the exposure effects of inhaled substances [30].

HepG2, Caco-2, A549 are the standard cell lines used in multi-organ on a chip to recapitulate various functions of the liver, intestine and lungs. The HepG2 and Caco-2 cells were cultured in a bioreactor with a cell culture insert and a polycarbonate cell microfluidic platform. The aim of the perfusion-based co-culture of HepG2 and Caco-2 cells was to assess the viability, integrity and functionality of the chip device. The comparison with the monoculture system suggested the improved biotransformation of the drugs exposed to the liver cells [29, 31].

Table 9.1 Various cell sources used for co-culture in multi-organ on a chip

MOC system	Source of cells	Application	Reference
Multi-organ microfluidic chip	• Human lung cancer cell line PC9 • Human bronchial epithelial cells (16HBE) • Human pulmonary microvascular endothelial cells (hPMEC) • Human lung fibroblasts (HFL1) • Human mononuclear cells (THP-1) • Human astrocytes (HA-1800) • Human brain microvascular endothelial cells (hBMVECs)	To recapitulate brain metastasis and BBB extravasation	[15]
Multi-organ-chip co-culture of liver and testis equivalents	• Differentiated HepaRG cells • Primary testicular tissue isolated from patients	To study systemic male reprotoxicity model	[16]
A pumpless, multi-organ-on-a-chip system	• Bone marrow-derived cells • Kasumi-1 myeloblasts MEG-01 megakaryocytes	To predict pre-clinical on-target efficacy, metabolic conversion and measurement of off-target toxicity of drugs using functional biological micro-electromechanical system	[17]
An integrated biomimetic array chip with co-cultured 3D liver and tumour microtissues	• Primary human hepatocytes (PHHs) • HepG2 cells • HUVECs • HCT116 cells • MCF7 cells • DU145 cells • U251 cells	Advanced anti-cancer bioactivity screening	[18]

(continued)

Table 9.1 (continued)

MOC system	Source of cells	Application	Reference
Microfluidic bilayer co-culture platform	• Primary human retinal microvascular endothelial cells (ECs) • Primary human dermal microvascular ECs • Immortalized human retinal pericytes (PCs)	To study endothelial–pericyte interactions and develop high-throughput PREDICT96 platform for microvascular co-culture model	[19]
A vascular-liver chip	• hPSC-derived hepatocytes (hPSC-HEPs) • hPSC-derived endothelial cells	Sensitive detection of nutraceutical metabolites from human pluripotent stem cell derivatives	[20]
Liver–brain chip	• Primary rat brain microvascular endothelial cells (BMECs) • Cerebral astrocytes • HepG2 (hepatoma cell line) cells • U87 cells (astrocytoma cell line)	Evaluation of hepatic drug metabolism for glioblastoma	[21]
Multi-organoids-on-chip system	• Human induced pluripotent stem cells (hiPSCs)	Safety assessment of antidepressant drugs	[22]
Alveolus on a chip	• Bronchial epithelial 16HBE14o− cells • Primary human alveolar epithelial cells (hAEpCs) • Primary human lung microvascular endothelial cells	Potential tool for lung research, drug discovery and precision medicine	[23]
Human pancreatic islets and liver spheroids on a chip	• Differentiated HepaRGs • Primary human hepatic stellate cells (HHSteCs) • Pancreatic islet microtissues	A novel human ex vivo type 2 diabetes model	[24]

(continued)

Table 9.1 (continued)

MOC system	Source of cells	Application	Reference
Human skin on a chip	• Human N/TERT-1 keratinocytes • Human primary foreskin-derived dermal fibroblasts	To recapitulate the structure and functionalities of human skin	[25]
Small intestine on a chip	• Human intestinal microvascular endothelial cells (HIMECs) • Endoscopic tissue biopsies	Tool for studying metabolism, nutrition, infection and drug pharmacokinetics, as well as personalized medicine	[26]

The gastrointestinal tract, being the most prominent surface exposed to the external environment, needs to be understood for its primary absorption of molecules entering the digestive system. Knowing the consequences of digestion of a chemical or drug and its interaction with the cells is crucial for toxicological assessment. The prediction of the pharmacokinetics of drugs by different interconnecting tissues based on the micro-total analysis systems was made using the culture of Caco-2, HepG2 and A549 cells. The system was utilized to mimic the organ–organ network involving a microfluidic network. The device was the first demonstration of a biological model with a top-down approach and was used to analyse the effects of orally administered drugs [32].

Another co-culture system involving the primary human intestinal epithelial cells (hIECs) and liver cells was developed to mimic the gastrointestinal tract-liver model for predicting the human responses in the pre-clinical studies. The viability of hIECs and HepG2 C3A cells was 14 days when cultured in a 3D polymer scaffold, and they formed a liver micro-lobe-like structure. The TEER values of the native gut were measured from the monolayer formed by hIECs on the polycarbonate membranes. The permeability of the hIECs was comparable to the conventionally used permeability models. The developed device was a low-cost GI-liver model [33].

To fabricate a physiologically relevant model of the GI tract, an in vitro microscale cell culture analogue (microCCA) of the GI tract was developed. Caco-2 and HT29-MTX cell lines were used in the system. The device mimics the systemic circulation coupled with a multi-chamber silicon microCCA. Caco-2 cells have microvilli and can produce tight junctions, while HT29-MTX mimics the goblet cells. Acetaminophen is used to analyse liver cell toxicity. The results were comparable with in vivo measurements in mice. The system was capable of understanding the potential toxicity of orally delivered drugs [34].

The prime objective of developing a multi-organ-on-a-chip system for drug development emerged from the idea of interconnecting several tissues and the perfusion of a specific common cell culture media. This system was also termed a

body on a chip as it was intended to recapitulate the reactions occurring in different tissue sites when a drug is introduced to the body. A body-on-a-chip design was developed for mimicking the human metabolism by providing a unidirectional flow of media through the organ chambers. The device was designed so that it permits the culture of each tissue separately and then combines them to form a functional body on a chip. The device was micro-fabricated using 3D printing. A polycarbonate membrane was sandwiched between two 3D printed polymers. Caco-2 cells were used for the GI tract construction, and 3D scaffolds were used to construct liver tissues. The primary human hepatocytes and a mixture of primary human fibroblasts, stellate cells, Kupffer cells, sinusoidal endothelial cells and vascular and biliary epithelial cells were seeded on the scaffold and kept in the organ chamber. The GI tract epithelium integrity, liver cell viability, albumin and urea production, CYP enzyme activity were assessed. All these data concluded that the viability and barrier function of the GI tract were retained for the 14-day co-culture period. The levels of aspartate aminotransferase were low throughout the culture, indicating low cell death levels [35].

9.3.3 Induced Pluripotent Stem Cells

The major drawback associated with the use of primary cells for the development of organ-on-a-chip devices is that they tend to alter their gene expression levels once they are removed from the parent organs. This led to induced pluripotent stem cells (iPSCs) for culturing in the organ-on-a-chip device. Induced pluripotent stem cells are somatic cells reprogrammed with various transcription factors and widely used in therapeutics. iPSCs can be the source of different human tissues. However, their potential applications are limited by their foetal-like nature in vitro culture systems [36]. These cells are used in OOC technology to develop a robust source of human cells for specific OOC models. Human-induced pluripotent stem cells (iPSCs) differentiated into BMEC-like cells (iBMECs) and microfluidics were combined to develop a human blood–brain barrier (BBB) model. The system utilized iPSC-derived brain microvascular endothelial-like cells (iBMECs), astrocytes and neurons to create the BBB model to predict the permeability of the blood–brain barrier. The iPSCs were seeded on collagen IV-fibronectin pre-coated channels of the chip. iBMECs were seeded on the blood side. Human neural cells (primary human astrocytes and pericytes) in the barrier side were co-cultured to analyse the cellular interactions and barrier functionality [37]. Another study utilized iPSC-derived cardiomyocytes and primary hepatocytes cultured in a multi-OOC to investigate the hepatic metabolism on off-target cardiotoxicity – the platform intended to study the in vivo cross-talk between the liver and heart. The heart liver systems were tested using specific drugs and compared with their metabolites in monoculture [38].

Human iPSCs were differentiated into spinal neural progenitor cells (spNPCs), subsequently studied in a spinal cord-chip system. Increased neuronal activity and expression of enhanced neuronal differentiation genes were identified in the spinal cord chip system compared with the cultures in 96 well plates. The inclusion of iPSC-derived BMECs resulted in a more in vivo like nature and increased neuronal

activity, vascular neural interaction induction. The spinal cord chip presents a novel perception of neuron-related diseases and the interaction of BMEC during early human development [39].

The use of iPSC is favoured for several reasons. The iPSC acts as a renewable cell source that could populate the OOC culture platforms, thereby avoiding the isolation of primary tissues from donors. The iPSCs could also closely mimic organ systems that could recapitulate the biological environment. This could be a better alternative for the pharmaceutical screening processes and the various clinical trials using OOC platforms that could emerge in the coming era of drug development. The standardization of protocols can also be improved by growing a common cell source that could differentiate into various cells. The integration of iPSCs as the major cell source in organ-on-a-chip technology would enhance the applicability of the technology for drug development, disease modelling and personalized medicine [40].

9.4 Cell Culture Techniques in MOC

Culture and co-culture techniques are essential to perform in vitro assessments of various cellular interactions. Various biological processes in human physiology, including development, homeostasis, diseases and regeneration, are studied using in vitro models and are anticipated as an alternative for animal models. Since the human body is a complex system consisting of many related functions, the co-culture in vitro models play a vital role in elucidating the interconnections between various tissues. The accomplishment of a perfect co-culture system depends on various factors like the composition of the media, the types of cells used and the ECM incorporated. Scaffold-based and scaffold-free culture techniques are used for the culture and co-culture of cells. The combination of microfluidic and 3D culture

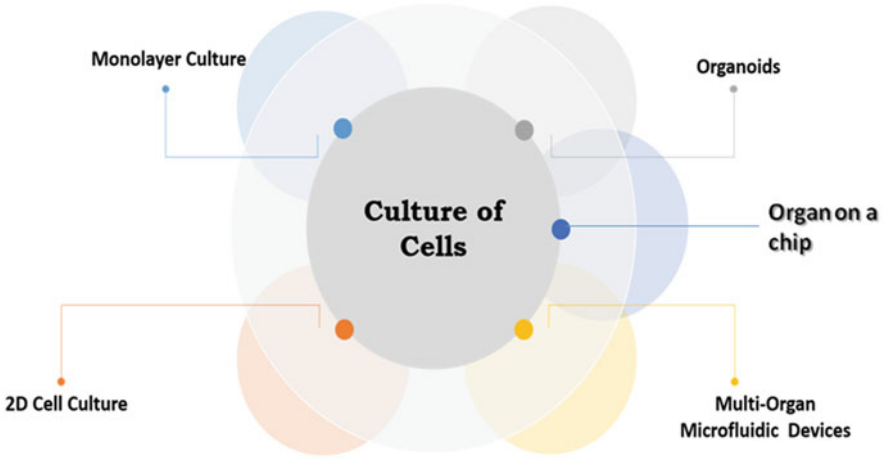

Fig. 9.2 Schematic representation of various types of cell culture

Table 9.2 Different membranes used for co-culture of cells

Membrane used	Cells used	Pore size	Application	Reference
Porous nanocrystalline silicon	3T3-L1 fibroblasts, primary endothelial cells (HUVEC)	0.003–0.008 μm pores	Feasibility of nanoporous membrane as a cell culture substrate	[41]
Silicon nitride	Astrocytes, immortalized brain capillary endothelial cell line SV-HCEC, HUVEC	0.27–0.7 μm pores	Blood–brain barrier	[42]
Silicon dioxide	Adipose-derived stem cells (ADSCs), HUVEC	0.5 and 3.0 μm pores	In vitro barrier and co-culture model	[43]
Polycarbonate	End3 endothelial cells, C8-D1A astrocytes	0.4 μm pores	Blood–brain barrier model	[44]
Polycarbonate (track etched)	Rat brain endothelial cell line (RBE4), E-18 rat cortical cells	0.8 μm pores	Neurovasculature	[45]
Polydimethyl siloxane	Human-induced pluripotent stem (hiPS), human glomerular endothelial cells	7 μm pores	In vitro model of the human kidney glomerulus	[46]

techniques provides real-time visualization of cellular processes through the OOC platforms. The schematics of various types of cell culture are shown in Fig. 9.2.

Multi-organ cell culture systems utilize porous semi-permeable membranes essential to construct cellular interfaces. The widely used porous membranes for co-culture are tabulated in Table 9.2. These porous membranes act as barrier models with established apical and basolateral surfaces and aids in the understanding of transportation of various molecules in and out of the cell and the barrier functions. The transport, secretion and absorption of smaller molecules can be easily measured when cultured in a membrane. Also, the migration of leukocytes in a cancer model can be studied. The integrated membrane models also support high-throughput screening by enhancing the imaging properties by providing a simple plane for the cells to attach [47]. The cells cultured on the opposite sides of a polycarbonate membrane can be moulded more precisely to the microfluidic flow conditions. They can be easily recovered from the devices than the hydrogel cultures.

Several factors are ruled by the physiological relevance of a co-culture system using a porous membrane for a barrier model. The membrane's pore size plays a significant role in determining the transmigrational properties, biochemical molecules exchange or cell-to-cell connections. Pore size, porosity and membrane thickness influence the permeability and movement of species from one

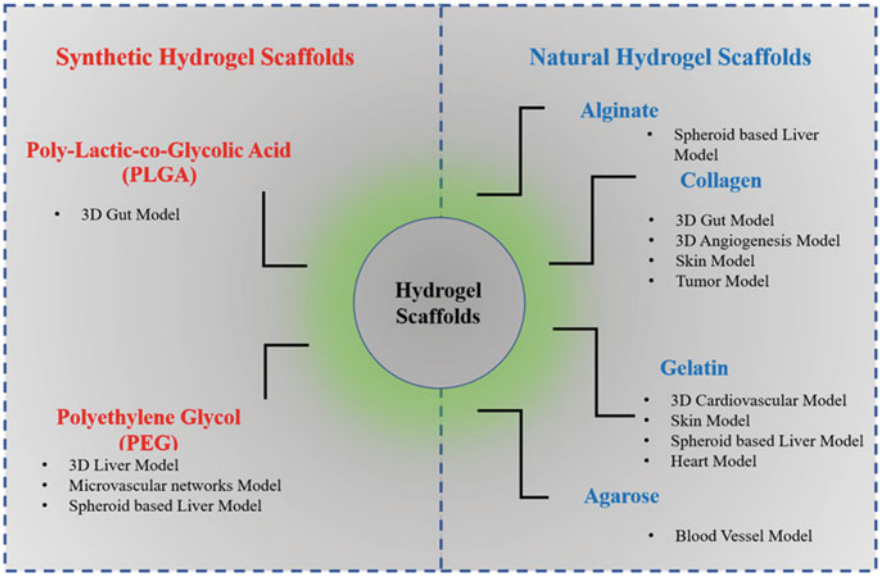

Fig. 9.3 Schematic representation of various hydrogel scaffolds used for cell culture

compartment to the next. The composition of the membrane can also affect the properties, and the attachment of the cells can be enhanced by modifications of the surface by methods such as plasma treatment or chemicals like APTES [48].

The limitations of conventional in vitro cell cultures raise the need for culture systems that can provide extracellular matrix support, thereby providing the in vivo like growth conditions for the cells. Many studies support the need for ECMs for in vitro cell cultures as they are crucial in maintaining cellular activities like proliferation, cell migration, response to chemical cues and apoptosis [49]. Accumulating pieces of evidence from the comparative studies of 2D and 3D cell cultures also reveals that the ECM plays a critical role in supporting and sustaining cellular functions [50]. Hence, the co-culture of cells in microfluidic devices and multi-organ on a chip using an extracellular matrix has been thoroughly studied. The primary scaffolds used for the co-culture of cells in multi-organ on a chip are hydrogels and electrospun fibres.

Hydrogel scaffolds are the most proffered scaffolds for cell culture as they provide in vivo like a matrix for the proper growth and development of the cells. Natural, synthetic and hybrid hydrogels are used as cell culture scaffolds as shown in Fig. 9.3. Collagen, alginate, chitosan, gelatin and fibrin are the most commonly used natural hydrogels. They are naturally present in the ECM and are highly biocompatible. However, the natural hydrogels suffer the limitations of long-term stability, poor mechanical properties and the variability of properties batch to batch [51].

The synthetic hydrogels like polyethene glycol (PEG) and its derivatives, including PEG-diacrylate (PEG-DA), polylactic acid (PLA), poly(lactic-*co*-glycolic acid)

(PLGA) and poly(ε-caprolactone) (PCL), are more advantageous in terms of stability and mechanical properties [52]. In OOC devices, hydrogels enhance the attachment of cells with the materials used for the fabrication of the devices. PDMS and Matrigel™ are the contemporary competitors used for the OOC devices to culture cells in a 3D environment. The PDMS, due to its elastic properties, allows the mechanical stretching of the cells as in the lung-on-a-chip devices but prevents the formation of natural ECM [53]. On the other hand, the gel-like scaffold sourced from animals, Matrigel™, enhances the growth and cellular attachment by the growth factors and hormones present in the scaffold [54].

A novel culture method for iPSC alveolar epithelial cells was developed using floating Matrigel droplets. The purified and sorted cells were embedded in a precursor form of liquid Matrigel. The cell-laden spheroids of Matrigel were changed to the media in spinning flasks, thereby forming floating droplets. This novel protocol was claimed to increase the proliferation of cells, thereby reducing the labour requirements of already-reported protocols [55].

9.5 Cell Culture in Multi-organ on a Chip

Human physiology is very complex and dynamic. The coordinated interaction of multiple organ systems is necessary to carry out all the body's functions. The complete understanding of various functions and their causes in the human body is not entirely understood yet. Any disintegration or miscommunication of even a tiny molecule can end up in the diseased condition of the body. The conventional 3D cell cultures that involve the growth of cells in an extracellular matrix have been shown to increase cellular functions and tissue organization [56]. The vital cellular functions like the organ-specific interactions and the spatiotemporal gradients of oxygen and chemicals are difficult to recapitulate even in the gel-based 3D cell culture models. The effects of the diffusion gradients of various chemicals and gases in the microenvironment are also tricky to capture in a gel-based culture.

The advancement of the single organ system directed the emergence of microphysiological systems with single interconnected organs or co-culture of multiple organs. This multi-organ-on-a chip (MOC) system or human-on-a-chip (HOC) system aids in the identification of dynamic and complex interactions between multiple organ models. It provides a better physiologically relevant understanding of the various cellular responses [57]. Hence, MOC can be a better alternative to understand the interaction of multiple organs or tissue systems and the onset of diseases. The utilization of MOCs to study pharmacokinetics and toxicokinetics can be an appreciable application.

The idea of OOC and multi-OOC (MOC) helps to bridge the gap between the physiological microenvironment and the cell culture models. With the incorporation of human-derived cells, the MOC devices can be an alternative to animal testing [58]. Compared to the conventional static cell culture systems, MOC closely resembles the human microenvironment and captures human physiology more closely. The potential applications of MOC in various fields, including drug

development, toxicity testing and personalized medicine, would improve the efficiency of in vitro models.

The major benefit of including micro-engineered techniques in cell cultures is that the extracellular matrix molecules in a 3D culture with the spatiotemporal gradients and other mechanical effects flourish in vivo conditions. Many studies reported that specially engineered substrates with specific micropatterns could induce cell growth in a three-dimensional manner and perform functions far better than the monolayer 2D cell systems. A muscular thin film was developed that allows cardiac cells to form a monolayer on a 2D substrate to enhance pharmacological stimulation [59]. The studies using engineered patterns for the cell cultures concluded that a mechanical environment is essential for cell growth attachment and differentiation. This also suggests that the flexibility of the extracellular matrix is highly essential for forming the cellular shape and for the cellular interactions that are anyhow not present in the conventional rigid culture systems. Hence, it is imperative to develop cell culture methods that would recapitulate the physical and chemical conditions of the microenvironments [54].

The integration of microfluidics and cell culture has witnessed the emergence of a novel platform that could enhance the scalability and reproducibility of in vitro culture systems. This allowed the systems to be drawn into micrometre levels, thereby enhancing organ function reproducibility. MOCs were developed for analysing the interaction of multiple organs when exposed to chemicals or drugs. The emergence of more sophisticated microfluidic-based multi-organ on a chip allowed the long-term culture of different types of cells and the engineering of more controlled environments. Several studies are reported with integrating two or more organ systems in a microfluidic setup to evaluate its toxicity and efficacy with the exposure of specific chemicals.

A liver and tumour model was combined to analyse the metabolism-dependent toxicity and anti-cancer efficacy. The hepatoma and colon cancer cells were cultured in a 3D hydrogel to recapitulate the multi-organ interactions. The cells were encapsulated in Matrigel cultures in different cell chambers and interconnected by channels that mimicked the blood flow [60]. A multilayer microfluidic device combining four different cell types, including liver cancer cells, lung cancer cells, breast carcinoma cells and normal gastric cells, was cultured in PDMS separated by polycarbonate porous membrane. This multi-organ device was used to decipher the drug-induced toxicity in various target tissues and metabolism-dependent drug efficacy as seen in in vivo environment [61].

The microfluidics cell culture has paved the way for revolutionary applications in drug development, tissue modelling and lab-on-a-chip technology. The fact that these technologies could far better mimic the in vivo environment from the conventional monolayer culture systems makes them the raw materials for next-generation cell culture practices that would provide physiologically relevant data and interpretations [62]. Microfluidic cell cultures or the OOC models incorporate the physical and biological interactions in the microenvironment, thereby providing a better prediction of human physiology. The OOC also provides long-term culture platforms where the time-dependent physiological responses could be recorded.

Many studies are being conducted on various OOC models for applications involving toxicity analysis, drug discovery and cancer studies.

Although the microfluidic cell culture systems are in their developing status, much research has already provided the basics and better understanding of various aspects of cell cultures in a microfluidic setup. The design of the microfluidic device is the primary factor contributing to the success of a microfluidic cell culture system. A primary factor affecting the microfluidic platform's cell culture is the culture volume. The controlled delivery and the movement of biomolecules in and out of the device and the mechanical forces in the fluid flow are all considered in a perfusion culture system [63].

The most popular OOC device developed so far is the alveolus on a chip or more precisely termed as the breathing lung on a chip owing to its recapitulation of the pulmonary oedema [64]. The organ consisted of two cells, human pulmonary microvascular endothelial cells and alveolar epithelial cells, cultured in parallel microfluidic channels with a porous membrane in between. The device could analyse the vascular leakage from the epithelial–endothelial intercellular space and the mechanical breathing motion of the lung-on-a-chip device. Another primary human lung alveolus-on-a-chip model was developed for analysing intravascular thrombosis [65]. The study mimicked the 3D cross-section of a human lung alveolus composed of an alveolar–capillary interface and a vascular lumen. Human umbilical vein endothelial cells (HUVECs) and primary human alveolar epithelial cells were cultured in the lower and upper channels separated by an ECM-coated porous membrane.

9.6 Conclusion

The above discussion focuses on the various cell culture techniques that can be integrated into a multi-organ-on-a-chip system that could elucidate the potential of a microfluidic approach to enhance the application of physiologically relevant 3D culture models. Over the past decade, the OOC culture platforms have evolved through a broad range of applications spanning the fields of drug discovery, toxicological studies, tissue engineering and disease modelling. The aim of developing a co-culture technique that could capture the in vivo human environment that would exist as an alternative for the animal models used in various experiments has seen many developments so far. Any system intended to mimic the human microphysiological environment must incorporate human tissues and cells in the system to provide the exact replication of the functions happening in the system. Therefore, the utilization of iPSCs could enhance the functioning and physiology of the co-culture systems intended to predict human physiology. The ability of iPSCs to differentiate into the various cell types provides an added advantage that can be utilized to recapitulate various disease models and trace the genotypic and phenotypic variations that occur in the cells.

The development of co-culture studies would provide promising applications and elaborate the understanding of cellular mechanisms that decipher inter-organ

communication. Incorporating microfluidics in recapitulating the micro-physiological environment provides an exciting and advantageous approach towards 3D culture techniques. The development of individual organ-on-a-chip devices cannot fully decipher the physiological functions since the tissue systems work interconnected. Communication of cells and tissues through different signaling pathways are an integral mechanism of all physiological functions occurring in a living system. There comes the importance of multi-organ cell cultures that integrate co-culture techniques and could elucidate the intracellular communications, thereby increasing the physiological relevance. Growing cells in microfluidic systems, especially in a co-culture manner, is an intensely difficult task as all the cells have to attain the same cellular density to be measured equally for the biochemical and mechanical signals. There have been various developments in the field of co-culture using microfluidics that contributed to the organ-on-a-chip technology for drug development and disease modelling. The co-culture techniques would aid in the drug interaction studies that can elevate the pre-clinical trials and drug discovery.

Understanding advanced co-culture techniques and the integrations of various scaffolds that can support the culture of more than one cell type could pave the way for developing integrated organ-on-a-chip or the 'human-on-a-chip' systems that could serve as the best alternative for experimental animal models. The analysis of drug side effects in the pre-clinical trials and patient responses could be more physiologically relevant than the animal models because of the use of human cells in the interconnected organ systems or the 'human-on-a-chip' systems. The development of various scaffolds that could support the growth of more than one cell type has been a prime area of research for tissue engineers and scientists. Moreover, there have been promising scaffold manufacturing techniques that could aid in developing functional multi-organ-on-chip systems. Nevertheless, many challenges need to be overcome to create a reliable and reproducible model of organ on a chip, and the standardization of a perfect scaffold would also need to be cross-checked.

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References

1. Langhans SA (2018) Three-dimensional in vitro cell culture models in drug discovery and drug repositioning. Front Pharmacol 9:6
2. Hussey GS, Dziki JL, Badylak SF (2018) Extracellular matrix-based materials for regenerative medicine. Nat Rev Mater 3:159–173
3. Goers L, Freemont P, Polizzi KM (2014) Co-culture systems and technologies: taking synthetic biology to the next level. J R Soc Interface 11:20140065
4. Liu X, Fang J, Huang S et al (2021) Tumor-on-a-chip: from bioinspired design to biomedical application. Microsyst Nanoeng 7:1–23

5. Sung JH, Wang YI, Narasimhan Sriram N et al (2018) Recent advances in body-on-a-chip systems. Anal Chem 91:330–351
6. Abulaiti M, Yalikun Y, Murata K et al (2020) Establishment of a heart-on-a-chip microdevice based on human iPS cells for the evaluation of human heart tissue function. Sci Rep 10:1–12
7. Jayne RK, Karakan MÇ, Zhang K et al (2021) Direct laser writing for cardiac tissue engineering: a microfluidic heart on a chip with integrated transducers. Lab Chip 21:1724–1737
8. Gijzen L, Yengej FAY, Schutgens F et al (2021) Culture and analysis of kidney tubuloids and perfused tubuloid cells-on-a-chip. Nat Protoc 16:2023–2050
9. Cohen A, Ioannidis K, Ehrlich A et al (2021) Mechanism and reversal of drug-induced nephrotoxicity on a chip. Sci Transl Med 13:eabd6299
10. Huang D, Liu T, Liao J et al (2021) Reversed-engineered human alveolar lung-on-a-chip model. Proc Natl Acad Sci U S A 118(19):e2016146118
11. Tas S, Rehnberg E, Bölükbas DA et al (2021) 3D printed lung on a chip device with a stretchable nanofibrous membrane for modeling ventilator induced lung injury. bioRxiv. https://doi.org/10.1101/2021.07.02.450873
12. Meng Q, Wang Y, Li Y, Shen C (2021) Hydrogel microfluidic-based liver-on-a-chip: mimicking the mass transfer and structural features of liver. Biotechnol Bioeng 118:612–621
13. Tian T, Chen C, Sun H et al (2021) A 3D bio-printed spheroids based perfusion in vitro liver on chip for drug toxicity assays. Chinese Chem Lett. https://doi.org/10.1016/j.cclet.2021.11.029
14. Shuler ML, Ghanem A, Quick D et al (1996) A self-regulating cell culture analog device to mimic animal and human toxicological responses. Biotechnol Bioeng 52:45–60
15. Liu W, Song J, Du X et al (2019) AKR1B10 (Aldo-keto reductase family 1 B10) promotes brain metastasis of lung cancer cells in a multi-organ microfluidic chip model. Acta Biomater 91:195–208. https://doi.org/10.1016/j.actbio.2019.04.053
16. Baert Y, Ruetschle I, Cools W et al (2020) A multi-organ-chip co-culture of liver and testis equivalents: a first step toward a systemic male reprotoxicity model. Hum Reprod 35:1029–1044
17. McAleer CW, Long CJ, Elbrecht D et al (2019) Multi-organ system for the evaluation of efficacy and off-target toxicity of anti-cancer therapeutics. Sci Transl Med 11(497):eaav1386
18. Hou Y, Ai X, Zhao L et al (2020) An integrated biomimetic array chip for high-throughput co-culture of liver and tumor microtissues for advanced anti-cancer bioactivity screening. Lab Chip 20:2482–2494
19. Rogers MT, Gard AL, Gaibler R et al (2021) A high-throughput microfluidic bilayer co-culture platform to study endothelial-pericyte interactions. Sci Rep 11:1–14
20. Yu F, Goh YT, Li H et al (2020) A vascular-liver chip for sensitive detection of nutraceutical metabolites from human pluripotent stem cell derivatives. Biomicrofluidics 14:034108
21. Li Z, Li D, Guo Y et al (2021) Evaluation of hepatic drug-metabolism for glioblastoma using liver-brain chip. Biotechnol Lett 43:383–392
22. Yin F, Zhang X, Wang L et al (2021) HiPSC-derived multi-organoids-on-chip system for safety assessment of antidepressant drugs. Lab Chip 21:571–581
23. Stucki JD, Hobi N, Galimov A et al (2018) Medium throughput breathing human primary cell alveolus-on-chip model. Sci Rep 8:1–13
24. Bauer S, Huldt CW, Kanebratt KP et al (2017) Functional coupling of human pancreatic islets and liver spheroids on-a-chip: towards a novel human ex vivo type 2 diabetes model. Sci Rep 7:1–11
25. Sriram G, Alberti M, Dancik Y et al (2018) Full-thickness human skin-on-chip with enhanced epidermal morphogenesis and barrier function. Mater Today 21:326–340
26. Kasendra M, Tovaglieri A, Sontheimer-Phelps A et al (2018) Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids. Sci Rep 8:1–14
27. Zhang C, Zhao Z, Rahim NAA et al (2009) Towards a human-on-chip: culturing multiple cell types on a chip with compartmentalized microenvironments. Lab Chip 9:3185–3192
28. Astashkina A, Mann B, Grainger DW (2012) A critical evaluation of in vitro cell culture models for high-throughput drug screening and toxicity. Pharmacol Ther 134:82–106

29. Prot JM, Maciel L, Bricks T et al (2014) First pass intestinal and liver metabolism of paracetamol in a microfluidic platform coupled with a mathematical modeling as a means of evaluating ADME processes in humans. Biotechnol Bioeng 111:2027–2040
30. Schimek K, Frentzel S, Luettich K et al (2020) Human multi-organ chip co-culture of bronchial lung culture and liver spheroids for substance exposure studies. Sci Rep 10:1–13
31. Bricks T, Paullier P, Legendre A et al (2014) Development of a new microfluidic platform integrating co-cultures of intestinal and liver cell lines. Toxicol In Vitro 28:885–895
32. Kimura H, Ikeda T, Nakayama H et al (2015) An on-chip small intestine–liver model for pharmacokinetic studies. J Lab Autom 20:265–273
33. Chen HJ, Miller P, Shuler ML (2018) A pumpless body-on-a-chip model using a primary culture of human intestinal cells and a 3D culture of liver cells. Lab Chip 18:2036–2046
34. Mahler GJ, Shuler ML, Glahn RP (2009) Characterization of Caco-2 and HT29-MTX co-cultures in an in vitro digestion/cell culture model used to predict iron bioavailability. J Nutr Biochem 20:494–502
35. Esch MB, Ueno H, Applegate DR, Shuler ML (2016) Modular, pumpless body-on-a-chip platform for the co-culture of GI tract epithelium and 3D primary liver tissue. Lab Chip 16: 2719–2729. https://doi.org/10.1039/C6LC00461J
36. Avior Y, Sagi I, Benvenisty N (2016) Pluripotent stem cells in disease modelling and drug discovery. Nat Rev Mol Cell Biol 17:170–182
37. Vatine GD, Barrile R, Workman MJ et al (2019) Human iPSC-derived blood-brain barrier chips enable disease modeling and personalized medicine applications. Cell Stem Cell 24:995–1005
38. Oleaga C, Riu A, Rothemund S et al (2018) Investigation of the effect of hepatic metabolism on off-target cardiotoxicity in a multi-organ human-on-a-chip system. Biomaterials 182:176–190
39. Sances S, Ho R, Vatine G et al (2018) Human iPSC-derived endothelial cells and microengineered organ-chip enhance neuronal development. Stem Cell Rep 10:1222–1236
40. Low LA, Tagle DA (2017) Organs-on-chips: progress, challenges, and future directions. Exp Biol Med 242:1573–1578
41. Agrawal AA, Nehilla BJ, Reisig KV et al (2010) Porous nanocrystalline silicon membranes as highly permeable and molecularly thin substrates for cell culture. Biomaterials 31:5408–5417
42. Ma SH, Lepak LA, Hussain RJ et al (2005) An endothelial and astrocyte co-culture model of the blood–brain barrier utilizing an ultra-thin, nanofabricated silicon nitride membrane. Lab Chip 5: 74–85
43. Carter RN, Casillo SM, Mazzocchi AR et al (2017) Ultrathin transparent membranes for cellular barrier and co-culture models. Biofabrication 9:15019
44. Booth R, Kim H (2012) Characterization of a microfluidic in vitro model of the blood-brain barrier (μBBB). Lab Chip 12:1784–1792. https://doi.org/10.1039/c2lc40094d
45. Achyuta AKH, Conway AJ, Crouse RB et al (2013) A modular approach to create a neurovascular unit-on-a-chip. Lab Chip 13:542–553. https://doi.org/10.1039/c2lc41033h
46. Musah S, Mammoto A, Ferrante TC et al (2017) Mature induced-pluripotent-stem-cell-derived human podocytes reconstitute kidney glomerular-capillary-wall function on a chip. Nat Biomed Eng 1:0069. https://doi.org/10.1038/s41551-017-0069
47. Zanella F, Lorens JB, Link W (2010) High content screening: seeing is believing. Trends Biotechnol 28:237–245
48. Ross AM, Jiang Z, Bastmeyer M, Lahann J (2012) Physical aspects of cell culture substrates: topography, roughness, and elasticity. Small 8:336–355
49. Hakkinen KM, Harunaga JS, Doyle AD, Yamada KM (2011) Direct comparisons of the morphology, migration, cell adhesions, and actin cytoskeleton of fibroblasts in four different three-dimensional extracellular matrices. Tissue Eng Part A 17:713–724
50. Chitcholtan K, Sykes PH, Evans JJ (2012) The resistance of intracellular mediators to doxorubicin and cisplatin are distinct in 3D and 2D endometrial cancer. J Transl Med 10:1–16
51. Giobbe GG, Crowley C, Luni C et al (2019) Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture. Nat Commun 10:1–14

52. Ding C, Chen X, Kang Q, Yan X (2020) Biomedical application of functional materials in organ-on-a-chip. Front Bioeng Biotechnol 8:823
53. Huh D (2015) A human breathing lung-on-a-chip. Ann Am Thorac Soc 12:S42–S44
54. Huh D, Hamilton GA, Ingber DE (2011) From 3D cell culture to organs-on-chips. Trends Cell Biol 21:745–754. https://doi.org/10.1016/j.tcb.2011.09.005
55. Becerra DC, Jeffs S, Wu T, Ott H (2021) High-throughput culture method of induced pluripotent stem cell derived alveolar epithelial cells using floating matrigel droplets. J Hear Lung Transplant 40:S52
56. Pampaloni F, Reynaud EG, Stelzer EHK (2007) The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 8:839–845
57. Sung JH (2021) Multi-organ-on-a-chip for pharmacokinetics and toxicokinetic study of drugs. Expert Opin Drug Metab Toxicol 17:969–986
58. Esch EW, Bahinski A, Huh D (2015) Organs-on-chips at the frontiers of drug discovery. Nat Rev Drug Discov 14:248–260
59. Alford PW, Feinberg AW, Sheehy SP, Parker KK (2010) Biohybrid thin films for measuring contractility in engineered cardiovascular muscle. Biomaterials 31:3613–3621
60. Sung JH, Shuler ML (2009) A micro cell culture analog (μCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs. Lab Chip 9:1385–1394
61. Li Z, Guo Y, Yu Y et al (2016) Assessment of metabolism-dependent drug efficacy and toxicity on a multilayer organs-on-a-chip. Integr Biol 8:1022–1029
62. D'Costa K, Kosic M, Lam A et al (2020) Biomaterials and culture systems for development of organoid and organ-on-a-chip models. Ann Biomed Eng 48:2002–2027
63. Kim L, Toh Y-C, Voldman J, Yu H (2007) A practical guide to microfluidic perfusion culture of adherent mammalian cells. Lab Chip 7:681–694
64. Huh D, Leslie DC, Matthews BD et al (2012) A human disease model of drug toxicity–induced pulmonary edema in a lung-on-a-chip microdevice. Sci Transl Med 4:159ra147
65. Jain A, Barrile R, van der Meer AD et al (2018) Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics. Clin Pharmacol Ther 103:332–340

Cells and Organs on a Chip in Biomedical Sciences

10

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Abstract

Advancement in lab-on-a-chip (LOC) and microfluidics technologies has led to the development of microscale cell culture platforms leading to physiological organ biomimetic systems built on microfluidic chips known as organ-on-chip (OOC). This integration of both electronic and fluidic components onto a single chip using a combination of bioengineering, cell biology and biomaterial technology has sparked research interest for newer applications in the biomedical sciences such as preclinical screening models for drug delivery systems, disease pathology, functioning of organs, 3D tissue engineering and point-of-care (PoC) diagnostics/treatment. In this chapter, we have mentioned the different types of OOC developed using microfabrication with living cells simulating tissue and organ cell physiology inside perfused chambers. The advancements as well as potential applications and future perspectives of these in vitro models of organs based on LOC devices which have been a major breakthrough for biological systems research have also been discussed. In the short term, OOCs can be an added advantage in traditional, preclinical cell culture methods at present and in vivo animal studies in the near future, and in the long term as replacements.

Keywords

Organ-on-a-chip · Diagnostics · Drug delivery · Treatment · Organ models · Tissue engineering · Microfluidics

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10.1 Introduction

The field of microfluidics involves precise manipulation and control of microfluid volumes (10^{-9} to 10^{-18} L) by using minute channels of diameter ranging from tens to hundreds of microns and is called a 'lab-on-a-chip' [1–3]. Due to the small size, high surface-to-volume ratio and large transfer of mass, microfluidics have the advantage of low and controllable sample/reagent volume, rapid mixing and response time and precise control over chemical and physical parameters [1, 4, 5]. Due to these unique properties, microfluidic technology can function as basic operating units like cell culture, sorting and cell lysis and hence can be applied in the development of organ-on-a-chip (OOC) [6, 7]. Since OOC combines multiple disciplines including bioengineering, cell biology, and biomaterial technology leading to various biomedical applications, it was entitled one of the 'Top Ten Emerging Technologies' at the World Economic Forum 2016. The basic aim of an OOC is to act as a biomimetic system that can simulate/mimic the physiological environment of human organs [8] and regulate cell patterning, shear force, tissue boundaries and tissue–organ interaction and concentration gradients [9–13].

The study of the human body and the functioning of its organ systems is significant to understand any pathological conditions and dysfunctions and is vital in the field of biomedical applications such as diagnostics, treatment, drug delivery, toxicology and disease pathogenesis [14]. The initial study of human physiology is carried out in traditional controlled 2D in vitro cell culture systems to study the interaction at lower levels such as genes, proteins, cells and tissues. However, 2D in vitro systems are unable to accurately simulate the complexity and physiological conditions of living organs, interaction of organ systems and microenvironmental factors [15, 16]. Hence, further validation via in vivo experiments that study animal model organisms is required. In vivo studies also face challenges of low throughput [17], inability to replicate human physiology due to species differentiation [18], high costs and ethical issues [19]. These challenges in preclinical testing of drugs are especially a major drawback mainly due to poor predictive power. Hence, OOC was proposed as the future technology to replace both in vitro and in vivo experimentation as a more efficient alternative for biomedical purposes by providing better physiological models [20, 21].

This chapter provides an overview of the different types of OOCs and multi-organ microdevices that have been developed using microfabrication technologies to mimic human physiology for various biomedical applications, mainly drug development and delivery, treatment, diagnostics and study of diseases. The key components required to develop OOCs, the designing concepts as well as the future prospective of this technology for cutting edge research in the field of biomedical sciences have also been discussed.

10.2 Key Components

The four main key components of OOCs are microfluidics, living cell tissues, stimulation or drug delivery and sensing [22]. The use of the microfluidic component is to deliver minute volumes of liquid to pre-designated target cells and involves liquid input and discharge units and can be characterized based on process automation, integration of units and miniaturization [6]. The type of cell spatially aligned in the 2-D/3-D system using biocompatible materials such as hydrogels makes up the second component. The biocompatible materials provide structure as well as reduce mechanical stress on the cells [23]. Certain tissues require a chemical/physical stimulant to kick-start tissue maturation and function such as myocardial tissues require an electrical signal to start maturing [24]. Hence, the third component involves various stimuli for the OOC to start. The final sensing component provides an embedded chip for output signal detection and data compilation to evaluate the organ system. For example, one research work provided phenotypes and statistical models of the cells [25], whereas another was able to monitor cellular activity in 3D microfluid using time-lapse microscopy [26]. An ideal OOC model requires sensing of the cellular metabolic states at specific time points.

10.3 Design Concept

For an OOC to be able to accurately simulate human physiological environment, both the external as well as internal environments of the cells need to be controlled keeping in mind different concepts during designing of the OOC such as fluid shear force, concentration gradients dynamic mechanical stress and cell patterning [8, 27].

10.3.1 Fluid Shear Force

To maintain cells in culture in an OOC, microfluidic-based micro-pump perfusion is used to provide nutrition and remove generated waste because the environment mimics in vivo environment rather than a static environment. This flow of fluid through microchannels results in fluid shear stress which in turn induces organ polarity [28]. This enables the OOC to exert necessary pressure on the functioning of cells by activating cell surface molecules and their associated signalling cascades [29]. Moreover, the fluid also enables specific monitoring at the single organ level [30]. The microfluidic flow may be a simple ‘rocker’ fluid motion or a more complex programmable ‘pulsatile’ fluid motion [31].

10.3.2 Concentration Gradient

Micro volumes of fluids primarily show the property of laminar flow and hence maintain an equal gradient of biomolecules both spatially and temporally. However,

in the case of human physiological environment, various biological processes including angiogenesis, invasion and migration require concentration gradient signalling [32–34]. Hence, to mimic this complex physiological environment similar to the human body, the velocity of the liquid and channel dimensions are altered using microvalves and micro-pumps to create biomolecule concentration gradients.

10.3.3 Dynamic Mechanical Stress

Within the human body, each organ undergoes different types of mechanical pressure such as blood pressure, lung pressure, and bone pressure which require maintenance of mechanically stressed tissues such as skeletal muscle, bone, cartilage and blood vessels [35–37]. In an OOC, microfluidic elastic porous membranes provide periodic mechanical stress to simulate the pressure in the body and is essential for differentiation [38, 39].

10.3.4 Cell Patterning

To form complete organs to simulate interactions similar to that in a human body, the arrangement of cells is very important. This complex geometric cell patterning is controlled using microfluidics which include surface modifications [40], templates [23] and 3D printing [41]. The 3-D structure is made using micro architectures [42] mostly consisting of hydrogel scaffolds and with multiple channels and 3D printing provides the advantage for versatile cell patterns based on the specific OOC to be developed. For example, Li et al. used controlled topological manipulations for heterotypic cell patterning on glass chips via polyvinyl acetate coating, carbon dioxide laser ablation and continuous cell seeding enabling epithelial–mesenchymal interactions [43].

10.4 Various Types of OOCs

Depending on the target organ model that needs to be studied, various OOCs based on different organs have been developed over the years to study specific organ pathology. Different organ OOCs have been listed in Table 10.1 along with the platform used and application.

10.4.1 Liver OOC

Since the liver is the main organ for drugs and toxin metabolism, it is essentially studied during drug developmental research. The liver is made up of multiple hepatic lobules which communicate for cellular function [92]. The main challenge of developing liver OOCs is maintaining the hepatocyte physiology over a long time

Table 10.1 Various types of OOCs characterized on the basis of platform used and application

S. No.	Organ	Platform	Application	Reference
1	Liver	Microfluidic bilayer	Drug screening and toxicology	[44]
2	Liver	Multi-well culture	Toxicology	[45]
3	Liver	Microfluidic perfused biochip	Human physiology	[46]
4	Liver	Bioreactor	Drug screening	[47]
5	Liver	Microfluidic multi-well array	Toxicology	[48]
6	Liver	Microfluidic endothelial-like barrier	Drug screening and toxicology	[49]
7	Liver	Microfluidic biochip	Morphology and viability study	[11]
8	Liver	Microwell PDMS multi-layer biochip	Long term perfusion culture	[50]
9	Liver	Microfabricated array bioreactor	Effect of hepatocytes on other cells and toxicology	[51]
10	Liver	Automated microfluidic bead-based electrochemical immunosensor	Detection and monitoring of cell secreted biomarkers	[52]
11	Liver	Microfluidics-based 3D dynamic cell culture system	Mimic native tumour microenvironment (TME) for cancer therapeutics	[53]
12	Liver	Single and dual microchannel configurations	Viral replication of hepatotropic hepatitis B virus (HBV)	[54]
13	Liver	Microfluidic co-cultures with integrated biosensors	Monitoring liver cell signalling molecules during alcohol injury	[55]
14	Lung	Biomimetic microsystem microdevice	Nanoparticle absorption uptake, transfer and nanotoxicology	[56]
15	Lung	Biomimetic microphysiological system	Environmental particulate screening and human pathophysiology	[57]
16	Lung	Small airway-on-a-chip	Lung pathophysiology	[58]
17	Lung	Biomimetic microfluidic device	Drug toxicity–induced pulmonary oedema study	[59]
18	Lung	Micro-diaphragm array (breathing-on-a-chip)	Study of mechanical strain induced by breathing	[60]
19	Lung	Microfluidic culture model	Responses of the airway epithelial barrier upon pollen exposure	[61]
20	Lung	Thermoplastic-based hydrogel microfluidic chip	Study of chronic lung diseases (CLDs)	[62]
21	Lung	Lung assisted device (LAD)	Impact of umbilical vessel expansion on vessel integrity for achieving large bore access	[63]

(continued)

Table 10.1 (continued)

S. No.	Organ	Platform	Application	Reference
22	Lung	Microfluidic oxygenator	Double-sided single oxygenator unit (dsSOU) LAD	[64]
23	Lung	Microfluidic chip-based 3D co-culture	Chemotherapeutic lung cancer drug testing	[65]
24	Heart	Cardiac electrophysiology and contraction-on-a-chip	Drug discovery and toxicology	[66]
25	Heart	Microfluidic biomimetic cardiac system	High-throughput pathophysiological studies and drug screening	[67]
26	Heart	PDMS based elastic muscular thin film (MTF)	Contractility assays for striated and smooth muscle	[68]
27	Heart	2D monolayer and 3D fibrin-based cardiac patch cultures	Drug development and cell therapy	[69]
28	Heart	Microfibrous hydrogel scaffolds and microfluidic perfusion bioreactor	Cardiovascular toxicology	[70]
29	Heart	High-speed impedance detection technology chip	Cardiac drug efficacy	[71]
30	Heart	Cell-laden gels and a pneumatic actuation system	Cardiac physiology	[72]
31	Heart	Multi-chamber system integrated device	Cardiac physiology	[73]
32	Kidney	Multi-layer microfluidic device	Drug screening and tissue engineering	[74]
33	Kidney	Glomerular capillary wall function-on-a-chip	Drug discovery and pathophysiology	[75]
34	Kidney	Proximal tubule-on-a-chip	Drug transport and nephrotoxicology	[76]
35	Kidney	Reusable microfluidic model	Renal physiology	[77]
36	Kidney	Tubuloids on a microfluidic plate	Study of infectious, malignant and hereditary kidney diseases	[78]
37	Kidney	Human islet organoids-on-a-chip	Stem cell-based engineering and drug development	[79]
38	Intestine	Biomimetic microdevice	Drug discovery and pathophysiology	[80]
39	Intestine	Primary human small intestine-on-a-chip	Pathophysiology, pharmacokinetics of drugs, personalized medicine	[81]
40	Intestine	Microfluidic gut-on-a-chip	Pathophysiology	[82]

(continued)

Table 10.1 (continued)

S. No.	Organ	Platform	Application	Reference
41	Intestine	Intestinal 3D organoid cultures	Pathophysiology	[35]
42	Brain	Organoid-on-a-chip	Study of prenatal nicotine exposure	[83]
43	Eye	Multi-layered microfluidic device	Tissue engineering	[84]
44	Eye	Retina-on-a-chip	Physiological studies	[85]
45	Eye	Biomimetic ocular models	Drug development	[86]
46	Skin	Skin-on-a-chip	Toxicology and pathophysiology	[87]
47	Skin	Perusable vascular channels-on-a-chip	Drug and cosmetic screening, pathophysiology	[88]
48	Bone	Bone angiogenesis model	Drug discovery, biomedical and toxicology	[89]
49	Bone marrow	Bone marrow-on-a-chip	Drug discovery, toxicology, tissue engineering and pathophysiology	[90]
50	Tumour	Microfluidic glioblastoma culture paradigm	Biology of brain tumours	[91]

[93]. The first liver OOC designed was made up of 3T3-J2 fibroblasts and rat hepatocytes (continuous stable synthesis of albumin while undergoing metabolism) co-cultured in microfluidic pores to simulate airway interface [48]. Another chip was designed to mirror the interstitial gap of endothelial cells and hepatocytes containing culture media which helped maintain exchange of substances [49]. In another novel technique to mimic the structure of hepatic lobules, radial electrophoretic field gradients were created onto circular polydimethylsiloxane (PDMS) chips [11]. In Fig. 10.1, preparation of decellularized liver matrixes (DLM) from native liver followed by fabrication of PDMS-based liver OOCs has been demonstrated.

Further advancement was carried out to develop 3D hepatocyte cultures using in situ perfusion of hepatic spheroids [50]. The effect of hepatocytes on other cells was studied through OOCs and liver cell toxicity assays were developed [51]. A microfluidic electrochemical-based biosensor was developed to detect hepatotoxicity biomarkers [52]. 3D liver tumour microenvironment (TME) was studied by integrating DLM with gelatin methacryloyl (GelMA) which can be further used in cancer therapeutics [53]. Furthermore, viral replication of the hepatitis B virus was studied [54] and an alcohol injury model to analyze liver injuries was developed using liver cell microsystems [55].

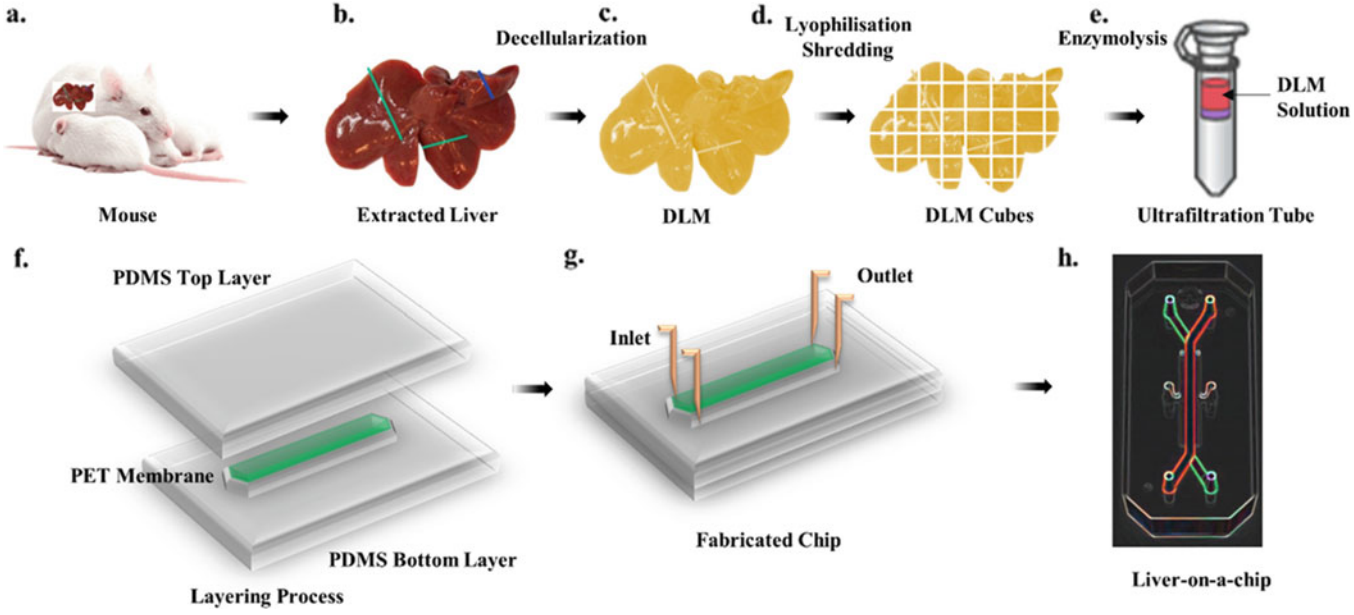

Fig. 10.1 Fabrication of Liver OOC: (**a**) Mouse/rat selected for natural liver sample; (**b**) Liver extracted from the animal model; (**c**) Decellularization of the extracted liver to form liver matrixes; (**d**) Lyophilization of the decellularized liver matrixes (DLM) into cubes; (**e**) Enzymolysis and ultrafiltration of the DLM solution to obtain liver cells required to fabricate the OOC; (**f**) Layering of a PET membrane with cultured liver cells sandwiched between and top and bottom PDMS layer; (**g**) Addition of nutrition inlet and waste disposal outlet channels to the fabricated chip; (**h**) Image of a fabricated PDMS based liver OOC

10.4.2 Lung OOC

Reproduction of lung alveoli in vitro where gas exchange takes place in the lungs is challenging. OOC microfluidics can enable development of extracorporeal lung models with gaseous exchange. The airway mechanical pressure, shear force and blood–brain barrier have been a major focus of study [94]. Soft lithography was used to develop a lung OOC model using 10 μm PDMS membranes separated by extracellular matrix (ECM) with an upper alveolar epithelial layer and lower human pulmonary microvascular endothelial layer simulating the alveolar–capillary barrier [56] as shown in Fig. 10.2 and respiratory expansion/contraction of the alveoli was simulated using vacuum and inflammation was caused using neutrophils as stimuli resulting in a pulmonary oedema model [59].

The first elastic model which could simulate breathing was developed by mimicking lung parenchyma [60]. In-depth study of the epithelial barrier was studied via 3D airway models with interstitial flow using a stent and permeable filter while the gas–liquid interface and dilation was simulated by applying shear force on the alveoli and capillaries [61]. Chronic lung disease model was developed by culturing epithelial cells on one side of a hydrogel membrane and smooth muscle cells on the other side [62]. Lung tissue OOCs can further be used as implantable respiratory medical devices. Lung assist devices (LAD) with high extra-corporeal blood flow for premature infants to provide gas exchange due to respiratory failure have been of immense help as clinical trials for umbilical vasodilation thresholds were unethical [63]. Umbilical vessel damage due to catheters could be now studied. Double-sided blood oxygenators were developed using OOC technology which showed 343% increase in uptake of oxygen as compared to single-sided oxygenators [64]. Chemotherapeutic lung cancer drugs were tested on lung cancer OOC models [65] while a recent study on asthma was carried out using a 'small airway-on-a-chip' [58].

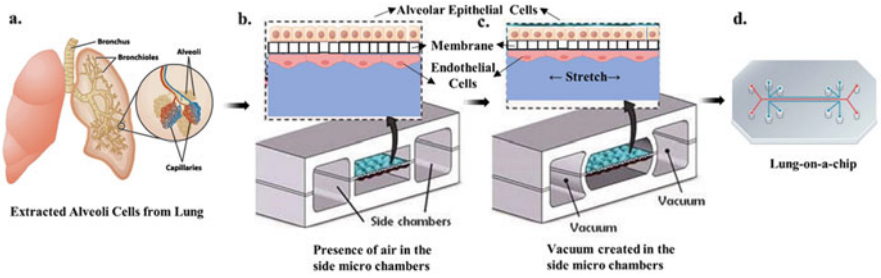

Fig. 10.2 Fabrication of Lung OOC: (**a**) Lung alveoli cells and capillary endothelial cells extracted to create a lung alveoli–capillary barrier simulation; (**b**) OOC fabricated using PDMS membranes separated by extracellular matrix (ECM) with an upper alveolar epithelial layer and lower human pulmonary microvascular endothelial layer where respiratory expansion was simulated by allowing air into the side microchannels and; (**c**) Respiratory contraction was simulation by introducing vacuum into the side microchannels resulting in stretching of cell layers; (**d**) Image of a fabricated lung alveoli–capillary barrier OOC simulating respiratory expansion and contraction via vacuum control

10.4.3 Heart OOC

The leading cause for mortality in the world is most often related to cardiovascular (CV) disorders but with the emergence of OOC technologies, in vitro bionic studies of the heart can now be carried out. With the myocardium being the main part of the cardiac system, pumping of cardiomyocytes (CMs) can be used as a marker to analyse the effect of drugs effects [67]. The contraction capacity of CMs was measured by the extent of curling of an elastic PDMS film implanted with neonatal rat CMs to simulate a muscle [68]. Subsequently, self-assembled myocardial sheets of CMs derived from differentiated myocardium were developed using hydrogel on PDMS [69]. 3D printing technology was later applied to integrate both the myocardial and vascular systems which created vascular networks for screening of CV drugs [70]. Preclinical studies of cardiac drug efficacy were carried out using high speed impedance on a heart OOC by recording CM contraction [71]. An OOC created to mimic CM microenvironment enabled direct imaging and visualization as well as low-cost quantitative analysis which could not be done using conventional cell culture or animal models [72]. Spatiotemporal pulsation dynamics of myocardial tissue along with its viability and functionality remaining stable for an extended time period was studied through optical detection on a fabricated OOC using human-induced pluripotent stem cells [73]. Cardiotoxicity is one of the major reasons for drug testing failure and hence these developed OOCs are important for drug development studies.

10.4.4 Kidney OOC

The kidney plays a major role in excretion of drugs from the body by maintaining osmotic pressure. Increased kidney toxicity can lead to irreversible damage of renal filtration which necessitates OOCs to test drug delivery. The main unit of the kidney are the nephrons which are made up of the glomerulus, renal capsule and renal tubule and are responsible for filtration and reabsorption. Microfluidic technology will enable growth of tubular cells, as well as maintenance of fluid environment and cell polarity [8]. The first multi-layered microfluidic OOC to simulate renal filtration was developed using mouse kidney medullary collecting duct cells which enhanced polarity of the medulla collecting duct by reorganization of the cytoskeleton and transport of molecules via hormonal stimuli [74] and the same device was later recreated using human primary renal epithelial cells (Fig. 10.3) for direct visualization of intact kidney tubules [76].

Nephrotoxicity assays, therapeutic studies, drug testing and kidney development and disease were carried out on OOCs mimicking glomerular capillary wall using pluripotent stem cell-derived podocytes [95]. The growth of renal epithelial cells under different conditions such as shear stress which can cause nephrotoxicity were studied using a reusable OOC consisting of human proximal tubules and glomeruli [77]. Stable tubular system was designed for expansion and kidney tissue analysis for applications such as disease modelling and drug screening [78]. Furthermore,

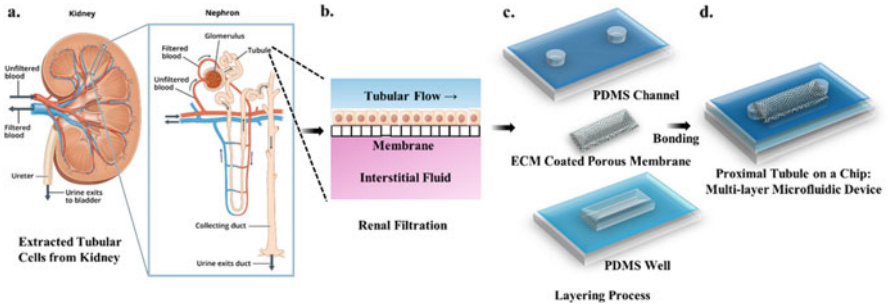

Fig. 10.3 Fabrication of Kidney OOC: (**a**) Kidney tubule cells extracted to create a human kidney renal filtration simulation; (**b**) OOC fabricated using PDMS membranes separated by extracellular matrix (ECM) with cultured tubular cells layer between tubular flow and interstitial fluid for filtration; (**c**) Layering of ECM coated porous membrane into the bottom PDMS layer containing a well and a top PDMS later containing inlet and outlet channels; (**d**) Fabricated proximal tubule on a chip simulating renal filtration

human islet organoids from human-induced pluripotent stem cells were generated for different applications such as stem cell-based engineering and drug development [79].

10.4.5 Intestine OOC

The need for different organs-on-chips having their own requirements and applications, associated with intestine, has always been strong. Whenever the intestine (small intestine and large intestine) is infected with bacteria or viruses, diagnosing it has always been a difficult task in the field of biomedical science. Wyss Institute at Harvard University has been the pioneer in development of in vitro OOCs and developed two layers gut on a chip system [80]. Intestine is a major site of commencing microbes of the gut microbiome to live and interact with gut lymphoid tissues and the host immune system, which helps maintain intestinal homeostasis [96, 97] and intestinal OOCs simulate this along with the peristaltic flow as shown in Fig. 10.4. Culturing an established human intestinal epithelial cell line is the most popular in vitro gut model used to study barrier function or model medication absorption on extracellular matrix (ECM)-coated porous membranes within Transwell insert culture devices. This 2-dimensional (2D) culture format fails to reproduce physiological 3-dimensional (3D) intestinal cell and tissue morphology or re-establish other critical intestine specialized functions, and is most typically used by the pharmaceutical industry [80, 98]. Several ex vivo models for drug transport assays have been developed, such as the everted sac18 or the Ussing chamber, however, their expected lifespan (less than 8 h) is insufficient to allow long-term studies on normal intestinal physiology, the development of intestinal disease models, or the study of clinically relevant host-microbiome crosstalk

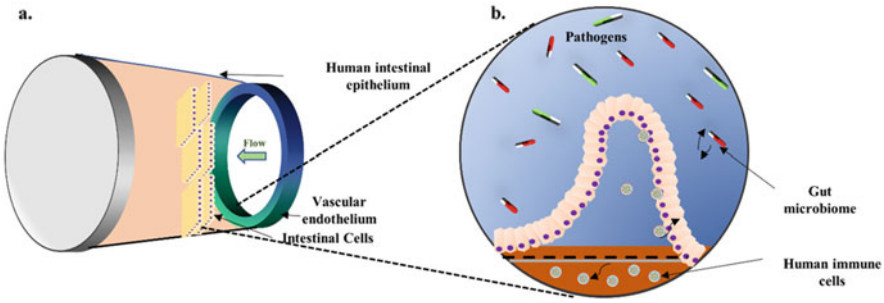

Fig. 10.4 Fabrication of Gut on a chip: (**a**) Simulation of fluid flowing through the intestine under peristalsis-like strain, between two layers of human villus intestinal epithelium and vascular endothelium lined on opposite sides of a flexible porous membrane; (**b**) The intestinal simulation shows complicated interplay between commensal gut microbiome, bacterial pathogens and human immune cells in parenchymal and vascular channels, respectively, as depicted in a higher magnification schematic

[99, 100]. Intestinal 3D organoid cultures derived from either intestinal crypts containing endogenous intestine cells or induced pluripotent stem cells have revolutionized the field by maintaining stem cell niches and supporting differentiation of various differentiated intestinal epithelial cell subtypes in vitro [101]. Small intestine organoids (enteroids) spontaneously undergo villus-crypt morphologic organization and intestinal histogenesis when cultivated in a 3D ECM gel in media containing Wnt, R-spondin, noggin and other growth factors [102]. Each organoid line derived from an individual patient's intestinal tissue biopsy can be grown, frozen and revived for multiple uses, potentially allowing biobanks to be established and multiplexed screening platforms to be developed for validating new drug candidates and advancing personalized medicine [103, 104]. The OOCs were originally created using processes adopted from computer microchip production (e.g. soft lithography) [8]. However, OOC models of the intestine have become more complex over the last 5 years, with neighbouring channels lined by human microvascular endothelium, commensal microbes, immune cells and pathogenic bacteria included, and some allowing the application of cyclic mechanical forces that mimic peristalsis-like deformations experienced by living intestine in vivo. Various types of created in vitro models that mimic the structure, function, physiology and pathology of the actual human intestine have been developed for implications of this research for future disease models, drug discovery and personalized medicine [105].

10.4.6 Brain OOC

The brain, which includes the central nervous system (CNS) and the spinal cord, is the most complicated organ in the human body. The brain, as the CNS's upper backbone, processes, integrates and coordinates information before making

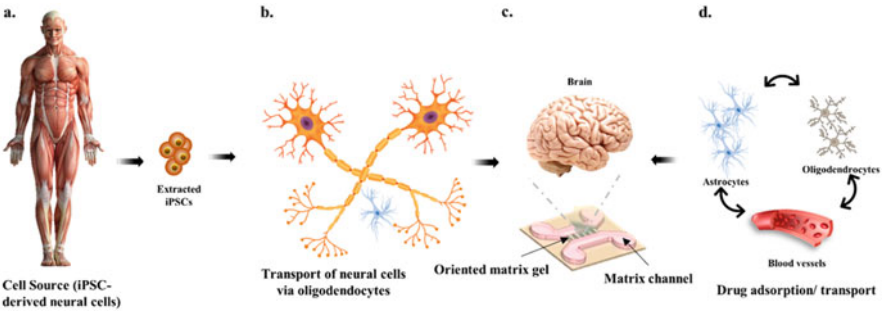

Fig. 10.5 Fabrication of brain on a chip: (**a**) Induced pluripotent stem cells derived neuronal cells isolated to be cultured on the OOC; (**b**) Isolated cortical neurons cultured onto an aligned extracellular matrix (ECM) in a segmented brain-on-a-chip system for modelling 3D neural networks; (**c**) A blood–brain barrier device on a chip comprising co-cultured brain endothelial cells and astrocytes in two channels separated by a porous membrane; (**d**) Adsorption and transport of signals from astrocytes, blood vessels and oligodendrocytes simulated on the fabricated brain OOC

decisions to organize the activity of different bodily organs. The brain is made up of many neurons that communicate with one another unidirectionally via synapses, where one cell's axon terminal touches the dendrites of another cell in a certain way [106]. The brain is composed of many aligned layers in which countless neuronal, glial and immune cells interact and are functionally shielded from toxicants by the strong blood–brain barrier and the skull from mechanical stress. Because the full structure and function of the brain in an engineered system is currently unavailable due to technical limitations, recent studies have focused on the recapitulation of specific parts of brain tissue, such as the unidirectional neural network, functionally tight blood–brain barrier, myelination process and spinal cord structure [107]. Neuronal cells have been cultivated in an open environment, such as glass substrates or Petri plates, since Harrison et al. developed the in vitro cell-culture technique in 1907 [108]. Whitesides et al. pioneered soft lithography, which allowed cell culture in a physically constrained microenvironment-like micro channels [109]. Later, utilizing the multi-step lithography approach, microchannels with varying heights and widths for a single brain on a chip were produced, allowing compartmentalized cultures to be performed by physically isolating the soma and axon and developing OOCs using induced pluripotent stem cells (iPSCs)-derived neural cells which simulate transport of signals from astrocytes, blood vessels and oligodendrocytes across the blood–brain barrier depicted in Fig. 10.5. The axon and soma of a neuron can be divided by creating high and low height microchannels within a single brain on a chip, allowing only the axon to travel through the shallow channels [110, 111]. This approach of 'compartmentalization' allows neuroscientists to analyse the features of the axon itself, treat medications just in the axonal region, or investigate axon regeneration following axotomy [112]. However, there are significant drawbacks to microchannel-mediated brain on chips, including 2D cell adhesion and the substantially stiffer mechanical qualities of glass substrates. Furthermore, unlike blood–

brain barrier, this monolayer structure cannot be employed to create a transport barrier.

Recent developments in brain on chips can be categorized into three categories: (1) 3D high-content systems that mimic the 3D brain tissue environment in terms of materials, cell types and physiological stimulation; (2) interconnected multi-chip systems that simulate cell-to-cell and organ-to-organ interactions; and (3) high-throughput systems that can massively screen various experimental conditions by making them compatible with traditional well plate-based assay systems. Although numerous brains on a chip models have been established, other elements such as cell sources, cell–cell interactions and cell–extracellular matrix (ECM) interactions must be considered in order to accurately simulate the structure and physiology of brain tissue. In terms of complexity and high-throughput/high-content screening capability, brain on a chip research has a long way to go. For individual research aims, such as axon-specific responses, cell–cell interactions and high-throughput screening, several brain on chip models are still commonly used [107].

10.4.7 Tumours on a Chip

Tumour tissues are made up of a variety of cell types (cancer cells, stromal cells such as cancer-associated fibroblasts, immune cells and vascular cells), as well as a lot of extracellular matrix components (i.e. type I collagen) [113–115]. The tumour stromal tissues (i.e. the tissues surrounding the tumour) operate as a dynamic source (and reservoir) of numerous cytokines and growth factors that influence tumour progression and pharmacological responses, in addition to their amazing internal heterogeneity. However, there are several compelling arguments in favour of using tumour-on-chip models as solid-tumour research tools. In a nutshell, these arguments revolve around three important properties of solid tumours: (a) their three-dimensional character, (b) their structural and dynamical complexity and (c) the fact that they are difficult to access and observe [116]. Traditional in vitro 3D cancer models are static systems (not based on microfluidics), but they are a valuable alternative to animal models and have enabled significant progress in cancer research over the last two decades [117–119]. While there are other methods developed for cancer diagnosis and imaging [120–126], microfluidics allows to keep and examine original or micro-fabricated tissue samples in a controlled environment while also reproducing (at least in part) the physiological dynamic properties [91]. Tumour-on-chip devices are predicted to enhance the discovery of novel and better cancer treatments while reducing non-specific toxicity [127–131]. Tumour-on-chip technologies are also finding useful niches in cancer research as essential tools [91] and have the potential to be strong enablers of anticancer personalized/precision medicine in the near future [132]. Tumour-on-chip technologies are currently the most promising in vitro platforms for authentically replicating relevant components of the biochemical complexity and dynamics of the tumour niche in a controlled setting [14, 133, 134].

10.4.8 Multi-organs-on-a-Chip

Multi-organ-on-a-chip (multi-OOC) devices have the potential to revolutionize how human health research is carried out [135]. Figure 10.6 depicts the different combinations of multiple organs which can be developed on a single chip for simultaneous multiple applications. Several in vitro cell culture systems are extensively utilized because they enable rapid drug discovery and disease modelling, as well as a controlled environment in which cellular development and activities can be

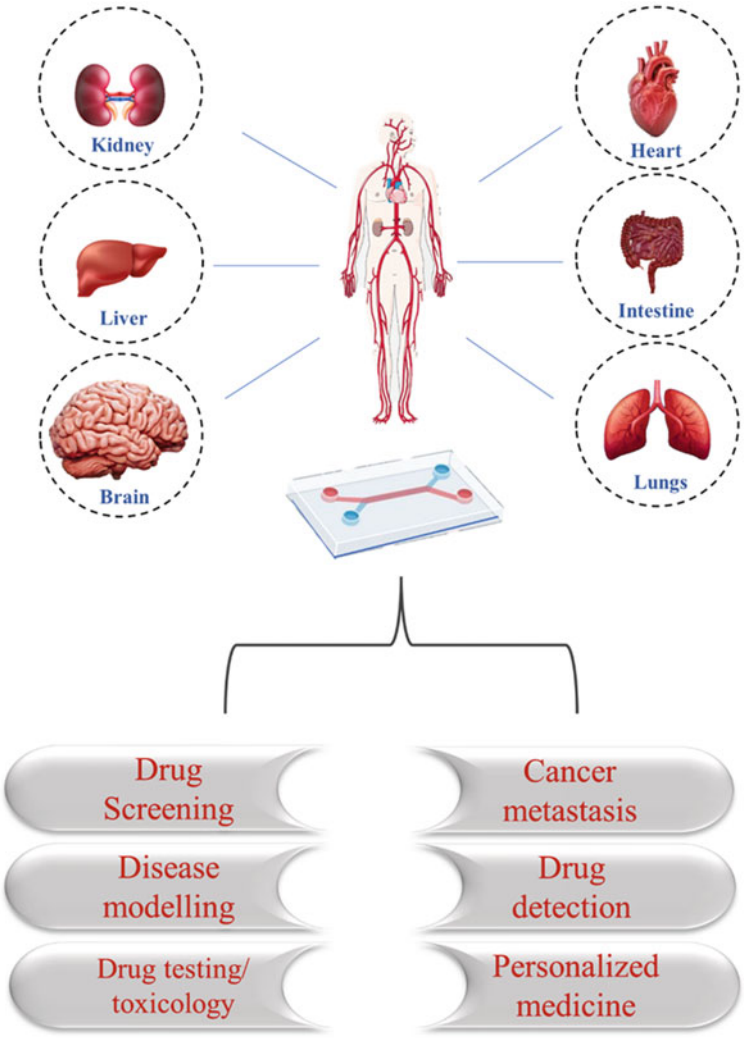

Fig. 10.6 Various multi-organ-on-a-chip combinations in different biomedical applications as a model of human physiology and pathophysiology

directly observed and tested [136]. The biochemical environment in vivo, as well as other mechanical features, is not replicated in typical 2D culture techniques, which allow cells to be grown in a monolayer. Furthermore, drug diffusion kinetics cannot be reliably exhibited in 2D cell cultures, because drug dosages that are successful in 2D invariably manifest as ineffectual in a real human body, and these culture models frequently lose their differentiated cell capabilities [137–140]. With increased tissue organization and enhanced expression of cell functions, 3D culture models have received attention in addressing the lack of physiological relevance, which is a key shortcoming of 2D cell cultures [141]. Animal models have also been hampered in their use as potent instruments for biological and pharmaceutical research due to various other limitations such as high cost and time, as well as ethical considerations [142].

Organ-on-a-chip (OOC) systems have recently emerged as alternatives to classic cell culture models, integrating cell culture with flow systems that simulate physiologically relevant circumstances and functionalities of organs. OOC systems are primarily based on microfluidic technology [31, 143–145]. Traditionally, many OOC models have been made out of polydimethylsiloxane (PDMS) elastomer, with UV lithography being used to produce the main chip architecture and soft lithography being used to generate an imprint of those structures to create microscale fluid channels. Because of its exceptional elasticity, the PDMS template allows more design flexibility for OOC models in this framework. Meanwhile, it can help to increase the adoption of commonly used optical measurement technologies and their integration with OOC systems [146]. However, these models have a few flaws, such as the need for multiple labour-intensive procedures and specialized equipment, which is costly and slows down design iterations, and the difficulty of simulating the intricate structures of the microenvironment in vivo [147]. Due to its processing versatility, rapid generation of microfluidic channels at a high efficiency, user-friendly equipment and significant methods that have been developed, using various natural bioinks, bioactive molecules and other bioactive molecules, 3D bioprinting technology has recently emerged as the most advanced technology for microfluidic device fabrication, and it has been applied to the development of OOC systems [148–150]. Furthermore, there are potential applications of multi-organ-on-a-chip in drug screening, cancer biomarkers detection and personalized medicine [151].

10.5 Applications and Challenges for Large-Scale OOC Implementation

The National Institutes of Health (NIH) and Defense Advanced Research Projects Agency (DARPA) studies began after major groundbreaking work in the fields of self-organizing organoids and early microphysiological systems (MPS) had been done [152–154]. The Advancing Regulatory Sciences initiative, a by-product of the joint NIH-FDA Leadership Council, was co-funded by the NIH and Food and Drug Administration (FDA) in 2010. The Harvard Wyss group's application to develop a 'Heart-Lung Micromachine' was one of the awarded programmes from this

initiative, and it led to pioneering work modelling the 'lung on a chip', then a DARPA, FDA and NIH workshop on the promise of MPS, and finally the launch of the NIH and DARPA MPS programmes in 2012. Since then, MPS programme grantees have created a wide range of platforms that mimic physiologically relevant in vitro settings, many of which are discussed in this chapter [56, 59, 155]. Advances in neural tissue engineering are among the program's other achievements. The Thomson/Murphy groups in Wisconsin, for example, have developed groundbreaking human embryonic stem cell-derived neural cell constructs that self-organize into in-vivo-like neural structures and are proving useful for fast predictive developmental neurotoxicity screening [156]. Blood vessel vasculature derived from iPSC-derived endothelial cells or blood endothelial progenitor cells, vascularized microtumours that recreate physiologically relevant vascularized tumorgenesis in vitro and enable dynamic interactions between tissues and tumours, and chemotherapeutic effects on healthy and cancerous tissues are among the Health MPS platforms developed within the programme [157–159]. These tissue-specific vascularization models will be useful as the scientific community clarifies the role of the endothelium lining of tissues, and microphysiological systems can provide crucial information to pave the way for more complicated tissue models.

A number of technical issues for the physical coupling of organ platforms are still being addressed. From linking human fallopian tube and ovarian tissue systems to developing integrated heart–liver–vasculature systems to collaborative work addressing the processes and challenges involved in functional and physical linkage of liver, gut, blood–brain barrier, kidney and vascular tissue platforms, NIH-funded teams have demonstrated successful functional integration of a number of systems [160, 161]. There are many distinct microfluidic platform designs, but each one is unique and, like the organ functions it represents, has varied flow rates that are acceptable for one organ system but not for others. Indeed, hepatic oxygen zonation in the liver may be mimicked solely by changes in cell medium flow rate inside the MPS platform, hence linking various organ systems necessitates complex technical solutions for controlled flow rates. For platform fabrication, several platforms use polydimethylsiloxane (PDMS), a transparent, flexible and inexpensive material. However, because PDMS is extremely lipophilic and binds a wide range of medicines and chemicals, the concentrations used on the platforms may need to be hundreds of times greater than those that eventually reach the tissues [162]. Despite the mixed species approach, these organ platforms have already showed considerable promise in terms of clinical relevance, with one major accomplishment being reprogramming the mouse ovary to cycle every 28 days, as opposed to every 9 days in mice [161].

10.6 Conclusion and Future Perspectives

OOCs are capable of simulating essential components of an in vivo human environment, including organ–organ interactions and the complex absorption, distribution, metabolism and elimination process. Herein we have reviewed the progress made in

realizing long-term testing of organ on chips in this chapter, which is critical for improving the capability of assessing multi-organ interactions more correctly and stably, and for better detection of chronic cellular reactions in the human body. The initial step toward achieving this goal was to use microfluidic technology to provide a constant and sustained flow of culture medium, as well as to connect distinct organ compartments for a long-term multi-tissue co-culture strategy. Biomedical sensors integrated into organ on chips systems enable low-cost, easy-to-use analytical platforms for detecting micro-environmental factors and electrophysiological responses for real-time monitoring of organ on chips platforms. Finally, building multi-sensor-integrated microfluidic organ on chips systems facilitates automatic and continuous monitoring of drug metabolic processes as well as organ on chips system parameters. However, there are still certain limits in the suggested experiments, and additional research on multi-sensor system integration would be necessary to overcome these concerns. These OOC applications demonstrated the ability to predict efficacy and harmful side effects with greater accuracy, facilitating medication development and the discovery of novel therapeutic techniques. Furthermore, we discussed various potential applications for long-term testing in organ-on-chips systems in chronic disease drug screening, cancer metastasis modelling for better understanding cancer biology and progress in drug discovery, biomarker detection for chronic organ responses and long-term pharmaceutical metabolism and personalized medicine for more accurate predictions.

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References

1. Whitesides GM (2006) The origins and the future of microfluidics. Nature 442:368–373. https://doi.org/10.1038/nature05058
2. Squires TM, Quake SR (2005) Microfluidics: fluid physics at the nanoliter scale. Rev Mod Phys 77:977–1026. https://doi.org/10.1103/RevModPhys.77.977
3. Mitchell P (2001) Microfluidics—downsizing large-scale biology. Nat Biotechnol 19:717–721. https://doi.org/10.1038/90754
4. Figeys D, Pinto D (2000) Lab-on-a-chip: a revolution in biological and medical sciences. Anal Chem 72:330–335. https://doi.org/10.1021/ac002800y
5. Haeberle S, Zengerle R (2007) Microfluidic platforms for lab-on-a-chip applications. Lab Chip 7:1094–1110. https://doi.org/10.1039/B706364B
6. Kwon JS, Oh JH (2018) Microfluidic technology for cell manipulation. Appl Sci 8:992–1011. https://doi.org/10.3390/app8060992
7. Sosa-Hernández JE, Villalba-Rodríguez AM, Romero-Castillo KD et al (2018) Organs-on-a-chip module: a review from the development and applications perspective. Micromachines 9:536–555. https://doi.org/10.3390/mi9100536

8. Bhatia SN, Ingber DE (2014) Microfluidic organs-on-chips. Nat Biotechnol 32:760–772. https://doi.org/10.1038/NBT.2989
9. Wang L, Liu W, Wang Y et al (2013) Construction of oxygen and chemical concentration gradients in a single microfluidic device for studying tumor cell-drug interactions in a dynamic hypoxia microenvironment. Lab Chip 13:695–705. https://doi.org/10.1039/c2lc40661f
10. Galie PA, Nguyen DHT, Choi CK et al (2014) Fluid shear stress threshold regulates angiogenic sprouting. Proc Natl Acad Sci U S A 111:7968–7973. https://doi.org/10.1073/pnas.1310842111
11. Ho CT, Lin RZ, Chen RJ et al (2013) Liver-cell patterning lab chip: mimicking the morphology of liver lobule tissue. Lab Chip 13:3578–3587. https://doi.org/10.1039/c3lc50402f
12. Booth R, Kim H (2012) Characterization of a microfluidic in vitro model of the blood-brain barrier (μBBB). Lab Chip 12:1784–1792. https://doi.org/10.1039/c2lc40094d
13. Sung JH, Shuler ML (2009) A micro cell culture analog (CCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs. Lab Chip 9:1385–1394. https://doi.org/10.1039/b901377f
14. Heylman C, Sobrino A, Shirure VS et al (2014) A strategy for integrating essential three-dimensional microphysiological systems of human organs for realistic anticancer drug screening. Exp Biol Med 239:1240–1254. https://doi.org/10.1177/1535370214525295
15. Jiang K, Dong C, Xu Y, Wang L (2016) Microfluidic-based biomimetic models for life science research. RSC Adv 6:26863–26873. https://doi.org/10.1039/c6ra05691a
16. Sung JH, Esch MB, Prot JM et al (2013) Microfabricated mammalian organ systems and their integration into models of whole animals and humans. Lab Chip 13:1201–1212. https://doi.org/10.1039/C3LC41017J
17. Halldorsson S, Lucumi E, Gómez-Sjöberg R, Fleming RMT (2015) Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices. Biosens Bioelectron 63:218–231. https://doi.org/10.1016/j.bios.2014.07.029
18. Van Der Meer AD, Van Den Berg A (2012) Organs-on-chips: breaking the in vitro impasse. Integr Biol 4:461–470. https://doi.org/10.1039/c2ib00176d
19. Al-Lamki RS, Bradley JR, Pober JS (2017) Human organ culture: updating the approach to bridge the gap from in vitro to in vivo in inflammation, cancer, and stem cell biology. Front Med 4:148–160. https://doi.org/10.3389/fmed.2017.00148
20. Lee SH, Jun BH (2019) Advances in dynamic microphysiological organ-on-a-chip: design principle and its biomedical application. J Ind Eng Chem 71:65–77. https://doi.org/10.1016/j.jiec.2018.11.041
21. Reardon S (2015) "Organs-on-chips" go mainstream. Nature 523:266. https://doi.org/10.1038/523266a
22. Mandenius CF (2018) Conceptual design of micro-bioreactors and organ-on-chips for studies of cell cultures. Bioengineering 5:56–77. https://doi.org/10.3390/bioengineering5030056
23. Tibbe MP, Leferink AM, van den Berg A et al (2018) Microfluidic gel patterning method by use of a temporary membrane for organ-on-chip applications. Adv Mater Technol 3:1–6. https://doi.org/10.1002/admt.201700200
24. Sun X, Nunes SS (2017) Maturation of human stem cell-derived cardiomyocytes in biowires using electrical stimulation. J Vis Exp 123:1–8. https://doi.org/10.3791/55373
25. Peel S, Corrigan AM, Ehrhardt B et al (2019) Introducing an automated high content confocal imaging approach for organs-on-chips. Lab Chip 19:410–421. https://doi.org/10.1039/c8lc00829a
26. Kane KIW, Moreno EL, Hachi S et al (2019) Automated microfluidic cell culture of stem cell derived dopaminergic neurons. Sci Rep 9:1–12. https://doi.org/10.1038/s41598-018-34828-3
27. Young EWK, Beebe DJ (2010) Fundamentals of microfluidic cell culture in controlled microenvironments. Chem Soc Rev 39:1036–1048. https://doi.org/10.1039/b909900j
28. Theobald J, Ghanem A, Wallisch P et al (2018) Liver-kidney-on-chip to study toxicity of drug metabolites. ACS Biomater Sci Eng 4:78–89. https://doi.org/10.1021/acsbiomaterials.7b00417

29. Davies PF (1995) Flow-mediated endothelial mechanotransduction. Physiol Rev 75:519–560. https://doi.org/10.1152/physrev.1995.75.3.519
30. Haddrick M, Simpson PB (2019) Organ-on-a-chip technology: turning its potential for clinical benefit into reality. Drug Discov Today 24:1217–1223. https://doi.org/10.1016/j.drudis.2019.03.011
31. Ronaldson-Bouchard K, Vunjak-Novakovic G (2018) Organs-on-a-chip: a fast track for engineered human tissues in drug development. Cell Stem Cell 22:310–324. https://doi.org/10.1016/J.STEM.2018.02.011
32. Yang KS, Cheng YC, Jeng MS et al (2014) An experimental investigation of micro pulsating heat pipes. Micromachines 5:385–395. https://doi.org/10.3390/mi5020385
33. Nguyen DHT, Stapleton SC, Yang MT et al (2013) Biomimetic model to reconstitute angiogenic sprouting morphogenesis in vitro. Proc Natl Acad Sci U S A 110:6712–6717. https://doi.org/10.1073/pnas.1221526110
34. Song JW, Daubriac J, Tse JM et al (2012) RhoA mediates flow-induced endothelial sprouting in a 3-D tissue analogue of angiogenesis. Lab Chip 12:5000–5006. https://doi.org/10.1039/c2lc40389g
35. Sato T, Clevers H (2013) Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. Science 340:1190–1194. https://doi.org/10.1126/science.1234852
36. Sellgren KL, Hawkins BT, Grego S (2015) An optically transparent membrane supports shear stress studies in a three-dimensional microfluidic neurovascular unit model. Biomicrofluidics 9:1–4. https://doi.org/10.1063/1.4935594
37. Yang SH, Choi JW, Huh D et al (2017) Roles of fluid shear stress and retinoic acid in the differentiation of primary cultured human podocytes. Exp Cell Res 354:48–56. https://doi.org/10.1016/j.yexcr.2017.03.026
38. Kshitiz PJ, Kim P et al (2012) Control of stem cell fate and function by engineering physical microenvironments. Integr Biol 4:1008–1018. https://doi.org/10.1039/c2ib20080e
39. Jang KJ, Cho HS, Kang DH et al (2011) Fluid-shear-stress-induced translocation of aquaporin-2 and reorganization of actin cytoskeleton in renal tubular epithelial cells. Integr Biol 3:134–141. https://doi.org/10.1039/c0ib00018c
40. Zhou J, Khodakov DA, Ellis AV, Voelcker NH (2012) Surface modification for PDMS-based microfluidic devices. Electrophoresis 33:89–104. https://doi.org/10.1002/elps.201100482
41. Xue D, Wang Y, Zhang J et al (2018) Projection-based 3D printing of cell patterning scaffolds with multiscale channels. ACS Appl Mater Interfaces 10:19428–19435. https://doi.org/10.1021/acsami.8b03867
42. Kaushik S, Gandhi S, Chauhan M et al (2020) Water-templated, polysaccharide-rich bioartificial 3D microarchitectures as extra-cellular matrix bioautomatons. ACS Appl Mater Interfaces 12:20912–20921. https://doi.org/10.1021/acsami.0c01012
43. Li YC, Lin MW, Yen MH et al (2015) Programmable laser-assisted surface microfabrication on a poly(vinyl alcohol)-coated glass chip with self-changing cell adhesivity for heterotypic cell patterning. ACS Appl Mater Interfaces 7:22322–22332. https://doi.org/10.1021/acsami.5b05978
44. Carraro A, Hsu WM, Kulig KM et al (2008) In vitro analysis of a hepatic device with intrinsic microvascular-based channels. Biomed Microdevices 10:795–805. https://doi.org/10.1007/s10544-008-9194-3
45. Khetani SR, Bhatia SN (2008) Microscale culture of human liver cells for drug development. Nat Biotechnol 26:120–126. https://doi.org/10.1038/nbt1361
46. Rennert K, Steinborn S, Gröger M et al (2015) A microfluidically perfused three dimensional human liver model. Biomaterials 71:119–131. https://doi.org/10.1016/j.biomaterials.2015.08.043
47. Knowlton S, Tasoglu S (2016) A bioprinted liver-on-a-chip for drug screening applications. Trends Biotechnol 34:681–682. https://doi.org/10.1016/j.tibtech.2016.05.014

48. Kane BJ, Zinner MJ, Yarmush ML, Toner M (2006) Liver-specific functional studies in a microfluidic array of primary mammalian hepatocytes. Anal Chem 78:4291–4298. https://doi.org/10.1021/ac051856v
49. Lee PJ, Hung PJ, Lee LP (2007) An artificial liver sinusoid with a microfluidic endothelial-like barrier for primary hepatocyte culture. Biotechnol Bioeng 97:1340–1346. https://doi.org/10.1002/bit.21360
50. Ma LD, Wang YT, Wang JR et al (2018) Design and fabrication of a liver-on-a-chip platform for convenient, highly efficient, and safe: in situ perfusion culture of 3D hepatic spheroids. Lab Chip 18:2547–2562. https://doi.org/10.1039/c8lc00333e
51. Yum K, Hong SG, Healy KE, Lee LP (2014) Physiologically relevant organs on chips. Biotechnol J 9:16–27. https://doi.org/10.1002/biot.201300187
52. Riahi R, Shaegh SAM, Ghaderi M et al (2016) Automated microfluidic platform of bead-based electrochemical immunosensor integrated with bioreactor for continual monitoring of cell secreted biomarkers. Sci Rep 6:1–14. https://doi.org/10.1038/srep24598
53. Lu S, Cuzzucoli F, Jiang J et al (2018) Development of a biomimetic liver tumor-on-a-chip model based on decellularized liver matrix for toxicity testing. Lab Chip 18:3379–3392. https://doi.org/10.1039/C8LC00852C
54. Kang YBA, Sodunke TR, Lamontagne J et al (2015) Liver sinusoid on a chip: long-term layered co-culture of primary rat hepatocytes and endothelial cells in microfluidic platforms. Biotechnol Bioeng 112:2571–2582. https://doi.org/10.1002/bit.25659
55. Zhou Q, Patel D, Kwa T et al (2015) Liver injury-on-a-chip: microfluidic co-cultures with integrated biosensors for monitoring liver cell signaling during injury. Lab Chip 15:4467–4478. https://doi.org/10.1039/c5lc00874c
56. Huh D, Matthews BD, Mammoto A et al (2010) Reconstituting organ-level lung functions on a chip. Science 328:1662–1668. https://doi.org/10.1126/SCIENCE.1188302
57. Huh D (2015) A human breathing lung-on-a-chip. Ann Am Thorac Soc 12:S42–S44. https://doi.org/10.1513/AnnalsATS.201410-442MG
58. Benam KH, Villenave R, Lucchesi C et al (2016) Small airway-on-a-chip enables analysis of human lung inflammation and drug responses in vitro. Nat Methods 13:151–157. https://doi.org/10.1038/nmeth.3697
59. Huh D, Leslie DC, Matthews BD et al (2012) A human disease model of drug toxicity-induced pulmonary edema in a lung-on-a-chip microdevice. Sci Transl Med 4:1–8. https://doi.org/10.1126/SCITRANSLMED.3004249
60. Stucki AO, Stucki JD, Hall SRR et al (2015) A lung-on-a-chip array with an integrated bio-inspired respiration mechanism. Lab Chip 15:1302–1310. https://doi.org/10.1039/c4lc01252f
61. Blume C, Reale R, Held M et al (2015) Temporal monitoring of differentiated human airway epithelial cells using microfluidics. PLoS One 10:1–13. https://doi.org/10.1371/journal.pone.0139872
62. Humayun M, Chow CW, Young EWK (2018) Microfluidic lung airway-on-a-chip with arrayable suspended gels for studying epithelial and smooth muscle cell interactions. Lab Chip 18:1298–1309. https://doi.org/10.1039/c7lc01357d
63. Peng J, Rochow N, Dabaghi M et al (2018) Postnatal dilatation of umbilical cord vessels and its impact on wall integrity: prerequisite for the artificial placenta. Int J Artif Organs 41:393–399. https://doi.org/10.1177/0391398818763663
64. Dabaghi M, Fusch G, Saraei N et al (2018) An artificial placenta type microfluidic blood oxygenator with double-sided gas transfer microchannels and its integration as a neonatal lung assist device. Biomicrofluidics 12:1–16. https://doi.org/10.1063/1.5034791
65. Xu Z, Gao Y, Hao Y et al (2013) Application of a microfluidic chip-based 3D co-culture to test drug sensitivity for individualized treatment of lung cancer. Biomaterials 34:4109–4117. https://doi.org/10.1016/j.biomaterials.2013.02.045

66. Qian F, Huang C, Lin YD et al (2017) Simultaneous electrical recording of cardiac electrophysiology and contraction on chip. Lab Chip 17:1732–1739. https://doi.org/10.1039/c7lc00210f
67. Visone R, Gilardi M, Marsano A et al (2016) Cardiac meets skeletal: what's new in microfluidic models for muscle tissue engineering. Molecules 21:1–21. https://doi.org/10.3390/molecules21091128
68. Grosberg A, Nesmith AP, Goss JA et al (2012) Muscle on a chip: in vitro contractility assays for smooth and striated muscle. J Pharmacol Toxicol Methods 65:126–135. https://doi.org/10.1016/j.vascn.2012.04.001
69. Zhang D, Shadrin IY, Lam J et al (2013) Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes. Biomaterials 34:5813–5820. https://doi.org/10.1016/j.biomaterials.2013.04.026
70. Zhang YS, Arneri A, Bersini S et al (2016) Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip. Biomaterials 110:45–59. https://doi.org/10.1016/j.biomaterials.2016.09.003
71. Zhang X, Wang T, Wang P, Hu N (2016) High-throughput assessment of drug cardiac safety using a high-speed impedance detection technology-based heart-on-a-chip. Micromachines 7:1–9. https://doi.org/10.3390/mi7070122
72. Marsano A, Conficconi C, Lemme M et al (2016) Beating heart on a chip: a novel microfluidic platform to generate functional 3D cardiac microtissues. Lab Chip 16:599–610. https://doi.org/10.1039/c5lc01356a
73. Schneider O, Zeifang L, Fuchs S et al (2019) User-friendly and parallelized generation of human induced pluripotent stem cell-derived microtissues in a centrifugal heart-on-a-chip. Tissue Eng A 25:1–33. https://doi.org/10.1089/ten.tea.2019.0002
74. Jang KJ, Suh KY (2010) A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells. Lab Chip 10:36–42. https://doi.org/10.1039/b907515a
75. Musah S, Mammoto A, Ferrante TC et al (2017) Mature induced-pluripotent-stem-cell-derived human podocytes reconstitute kidney glomerular-capillary-wall function on a chip. Nat Biomed Eng 1:1–12. https://doi.org/10.1038/s41551-017-0069
76. Jang KJ, Mehr AP, Hamilton GA et al (2013) Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment. Integr Biol 5:1119–1129. https://doi.org/10.1039/c3ib40049b
77. Sakolish CM, Philip B, Mahler GJ (2019) A human proximal tubule-on-a-chip to study renal disease and toxicity. Biomicrofluidics 13:1–12. https://doi.org/10.1063/1.5083138
78. Schutgens F, Rookmaaker MB, Margaritis T et al (2019) Tubuloids derived from human adult kidney and urine for personalized disease modeling. Nat Biotechnol 37:303–313. https://doi.org/10.1038/s41587-019-0048-8
79. Nieskens TTG, Sjögren AK (2019) Emerging in vitro systems to screen and predict drug-induced kidney toxicity. Semin Nephrol 39:215–226. https://doi.org/10.1016/j.semnephrol.2018.12.009
80. Kim HJ, Huh D, Hamilton G, Ingber DE (2012) Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip 12:2165–2174. https://doi.org/10.1039/C2LC40074J
81. Kasendra M, Tovaglieri A, Sontheimer-Phelps A et al (2018) Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids. Sci Rep 8:1–14. https://doi.org/10.1038/s41598-018-21201-7
82. Shim KY, Lee D, Han J et al (2017) Microfluidic gut-on-a-chip with three-dimensional villi structure. Biomed Microdevices 19:1–10. https://doi.org/10.1007/s10544-017-0179-y
83. Wang Y, Wang L, Zhu Y, Qin J (2018) Human brain organoid-on-a-chip to model prenatal nicotine exposure. Lab Chip 18:851–860. https://doi.org/10.1039/c7lc01084b
84. Puleo CM, McIntosh Ambrose W, Takezawa T et al (2009) Integration and application of vitrified collagen in multilayered microfluidic devices for corneal microtissue culture. Lab Chip 9:3221–3227. https://doi.org/10.1039/b908332d

85. Dodson KH, Echevarria FD, Li D et al (2015) Retina-on-a-chip: a microfluidic platform for point access signaling studies. Biomed Microdevices 17:1–10. https://doi.org/10.1007/s10544-015-0019-x
86. Estlack Z, Bennet D, Reid T, Kim J (2017) Microengineered biomimetic ocular models for ophthalmological drug development. Lab Chip 17:1539–1551. https://doi.org/10.1039/C7LC00112F
87. Wufuer M, Lee GH, Hur W et al (2016) Skin-on-a-chip model simulating inflammation, edema and drug-based treatment. Sci Rep 6:1–12. https://doi.org/10.1038/srep37471
88. Mori N, Morimoto Y, Takeuchi S (2017) Skin integrated with perfusable vascular channels on a chip. Biomaterials 116:48–56. https://doi.org/10.1016/j.biomaterials.2016.11.031
89. Jusoh N, Oh S, Kim S et al (2015) Microfluidic vascularized bone tissue model with hydroxyapatite-incorporated extracellular matrix. Lab Chip 15:48–56. https://doi.org/10.1039/c5lc00698h
90. Torisawa YS, Spina CS, Mammoto T et al (2014) Bone marrow-on-a-chip replicates hematopoietic niche physiology in vitro. Nat Methods 11:663–669. https://doi.org/10.1038/nmeth.2938
91. Olubajo F, Achawal S, Greenman J (2020) Development of a microfluidic culture paradigm for ex vivo maintenance of human glioblastoma tissue: a new glioblastoma model? Transl Oncol 13:1–10. https://doi.org/10.1016/J.TRANON.2019.09.002
92. McCuskey RS (2008) The hepatic microvascular system in health and its response to toxicants. Anat Rec 291:661–671. https://doi.org/10.1002/ar.20663
93. Cho CH, Park J, Tilles AW et al (2010) Layered patterning of hepatocytes in co-culture systems using microfabricated stencils. BioTechniques 48:47–52. https://doi.org/10.2144/000113317
94. Guenat OT, Berthiaume F (2018) Incorporating mechanical strain in organs-on-a-chip: lung and skin. Biomicrofluidics 12:1–15. https://doi.org/10.1063/1.5024895
95. Musah S, Dimitrakakis N, Camacho DM et al (2018) Directed differentiation of human induced pluripotent stem cells into mature kidney podocytes and establishment of a Glomerulus Chip. Nat Protoc 13:1662–1685. https://doi.org/10.1038/s41596-018-0007-8
96. Garrett WS, Mellman I (2001) Studies of endocytosis. In: Dendritic cells. Elsevier, Amsterdam, p 213
97. Round JL, Mazmanian SK (2009) The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9:313–323. https://doi.org/10.1038/NRI2515
98. Kim HJ, Ingber DE (2013) Gut-on-a-Chip microenvironment induces human intestinal cells to undergo villus differentiation. Integr Biol 5:1130–1140. https://doi.org/10.1039/C3IB40126J
99. Rozehnal V, Nakai D, Hoepner U et al (2012) Human small intestinal and colonic tissue mounted in the Ussing chamber as a tool for characterizing the intestinal absorption of drugs. Eur J Pharm Sci 46:367–373. https://doi.org/10.1016/J.EJPS.2012.02.025
100. Smith P, Mirabelli C, Fondacaro J et al (1988) Intestinal 5-fluorouracil absorption: use of Ussing chambers to assess transport and metabolism. Pharm Res 5:598–603. https://doi.org/10.1023/A:1015950215230
101. Sato T, van Es JH, Snipert HJ et al (2011) Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature 469:415–418. https://doi.org/10.1038/NATURE09637
102. Jung P, Sato T, Merlos-Suárez A et al (2011) Isolation and in vitro expansion of human colonic stem cells. Nat Med 17:1225–1227. https://doi.org/10.1038/NM.2470
103. van de Wetering M, Francies HE, Francis JM et al (2015) Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 161:933–945. https://doi.org/10.1016/J.CELL.2015.03.053
104. Sato T, Clevers H (2015) SnapShot: growing organoids from stem cells. Cell 161:1700–1700.e1. https://doi.org/10.1016/J.CELL.2015.06.028
105. Bein A, Shin W, Jalili-Firoozinezhad S et al (2018) Microfluidic organ-on-a-chip models of human intestine. Cell Mol Gastroenterol Hepatol 5:659–668. https://doi.org/10.1016/J.JCMGH.2017.12.010

106. Swanson LW, Lichtman JW (2016) From Cajal to connectome and beyond. Annu Rev Neurosci 39:197–216. https://doi.org/10.1146/ANNUREV-NEURO-071714-033954
107. Bang S, Jeong S, Choi N, Kim HN (2019) Brain-on-a-chip: a history of development and future perspective. Biomicrofluidics 13:1–7. https://doi.org/10.1063/1.5120555
108. Harrison RG (1906) Observations on the living developing nerve fiber. Soc Exp Biol Med 4: 140–143. https://doi.org/10.3181/00379727-4-98
109. Whitesides GM, Ostuni E, Takayama S et al (2001) Soft lithography in biology and biochemistry. Annu Rev Biomed Eng 3:335–373. https://doi.org/10.1146/ANNUREV.BIOENG.3.1.335
110. Park J, Koito H, Li J, Han A (2009) Microfluidic compartmentalized co-culture platform for CNS axon myelination research. Biomed Microdevices 11:1145–1153. https://doi.org/10.1007/S10544-009-9331-7
111. Taylor AM, Blurton-Jones M, Rhee SW et al (2005) A microfluidic culture platform for CNS axonal injury, regeneration and transport. Nat Methods 2:599–605. https://doi.org/10.1038/NMETH777
112. Tong Z, Segura-Feliu M, Seira O et al (2015) A microfluidic neuronal platform for neuron axotomy and controlled regenerative studies. RSC Adv 5:73457–73466. https://doi.org/10.1039/C5RA11522A
113. Kim J-Y, Fluri DA, Marchan R et al (2015) 3D spherical microtissues and microfluidic technology for multi-tissue experiments and analysis. J Biotechnol 205:24–35. https://doi.org/10.1016/J.JBIOTEC.2015.01.003
114. Erkan M, Hausmann S, Michalski CW et al (2012) The role of stroma in pancreatic cancer: diagnostic and therapeutic implications. Nat Rev Gastroenterol Hepatol 9:454–467. https://doi.org/10.1038/NRGASTRO.2012.115
115. Erdogan B, Ao M, White LM et al (2017) Cancer-associated fibroblasts promote directional cancer cell migration by aligning fibronectin. J Cell Biol 216:3799–3816. https://doi.org/10.1083/JCB.201704053
116. Santiago GT, Flores-Garza BG, Tavares-Negrete JA et al (2019) The tumor-on-chip: recent advances in the development of microfluidic systems to recapitulate the physiology of solid tumors. Materials 12:1–40. https://doi.org/10.3390/MA12182945
117. Katt ME, Placone AL, Wong AD et al (2016) In vitro tumor models: advantages, disadvantages, variables, and selecting the right platform. Front Bioeng Biotechnol 4:1–14. https://doi.org/10.3389/FBIOE.2016.00012
118. Verjans ET, Doijen J, Luyten W et al (2018) Three-dimensional cell culture models for anticancer drug screening: worth the effort? J Cell Physiol 233:2993–3003. https://doi.org/10.1002/JCP.26052
119. Gentile SD, Kourouklis AP, Ryoo H, Underhill GH (2020) Integration of hydrogel microparticles with three-dimensional liver progenitor cell spheroids. Front Bioeng Biotechnol 8:1–10. https://doi.org/10.3389/FBIOE.2020.00792
120. Shahdeo D, Chandra AB, Gandhi S (2021) Urokinase plasminogen activator receptor-mediated targeting of a stable nanocomplex coupled with specific peptides for imaging of cancer. Anal Chem 93:11868–11877. https://doi.org/10.1021/ACS.ANALCHEM.1C02697
121. Shahdeo D, Kesarwani V, Suhag D et al (2021) Self-assembled chitosan polymer intercalating peptide functionalized gold nanoparticles as nanoprobe for efficient imaging of urokinase plasminogen activator receptor in cancer diagnostics. Carbohydr Polym 266:1–10. https://doi.org/10.1016/j.carbpol.2021.118138
122. Chouhan RS, Horvat M, Ahmed J et al (2021) Magnetic nanoparticles—a multifunctional potential agent for diagnosis and therapy. Cancers 13:1–16. https://doi.org/10.3390/cancers13092213
123. Roberts A, Tripathi PP, Gandhi S (2019) Graphene nanosheets as an electric mediator for ultrafast sensing of urokinase plasminogen activator receptor-a biomarker of cancer. Biosens Bioelectron 141:1–8. https://doi.org/10.1016/J.BIOS.2019.111398

124. Tripathi PP, Arami H, Banga I et al (2018) Cell penetrating peptides in preclinical and clinical cancer diagnosis and therapy. Oncotarget 9:37252–37267. https://doi.org/10.18632/oncotarget.26442
125. Shahdeo D, Gandhi S (2022) Next generation biosensors as a cancer diagnostic tool. In: Biosensor based advanced cancer diagnostics. Elsevier, Amsterdam, pp 179–196. https://doi.org/10.1016/B978-0-12-823424-2.00016-8
126. Narlawar S, Coudhury S, Gandhi S (2022) Magnetic properties-based biosensors for early detection of cancer. In: Biosensor based advanced cancer diagnostics. Elsevier, Amsterdam, pp 165–178. https://doi.org/10.1016/B978-0-12-823424-2.00010-7
127. Sontheimer-Phelps A, Hassell BA, Ingber DE (2019) Modelling cancer in microfluidic human organs-on-chips. Nat Rev Cancer 19:65–81. https://doi.org/10.1038/S41568-018-0104-6
128. Tsai H-F, Trubelja A, Shen AQ, Bao G (2017) Tumour-on-a-chip: microfluidic models of tumour morphology, growth and microenvironment. J R Soc Interface 14:1–20. https://doi.org/10.1098/RSIF.2017.0137
129. Sun W, Luo Z, Lee J et al (2019) Organ-on-a-chip for cancer and immune organs modeling. Adv Healthc Mater 8:1–12. https://doi.org/10.1002/ADHM.201801363
130. Shang M, Soon RH, Lim CT et al (2019) Microfluidic modelling of the tumor microenvironment for anti-cancer drug development. Lab Chip 19:369–386. https://doi.org/10.1039/C8LC00970H
131. Caballero D, Kaushik S, Correlo VM et al (2017) Organ-on-chip models of cancer metastasis for future personalized medicine: from chip to the patient. Biomaterials 149:98–115. https://doi.org/10.1016/J.BIOMATERIALS.2017.10.005
132. van den Berg A, Mummery CL, Passier R, van der Meer AD (2019) Personalised organs-on-chips: functional testing for precision medicine. Lab Chip 19:198–205. https://doi.org/10.1039/C8LC00827B
133. Mannino RG, Santiago-Miranda AN, Pradhan P et al (2017) 3D microvascular model recapitulates the diffuse large B-cell lymphoma tumor microenvironment in vitro. Lab Chip 17:407–414. https://doi.org/10.1039/C6LC01204C
134. Zhang YS, Zhang Y-N, Zhang W (2017) Cancer-on-a-chip systems at the frontier of nanomedicine. Drug Discov Today 22:1392–1399. https://doi.org/10.1016/J.DRUDIS.2017.03.011
135. Paniel N, Radoi A, Marty JL (2010) Development of an electrochemical biosensor for the detection of aflatoxin M1 in Milk. Sensors 10:9439–9448. https://doi.org/10.3390/s101009439
136. Avior Y, Sagi I, Benvenisty N (2016) Pluripotent stem cells in disease modelling and drug discovery. Nat Rev Mol Cell Biol 17:170–182. https://doi.org/10.1038/NRM.2015.27
137. Huh D, Hamilton GA, Ingber DE (2011) From 3D cell culture to organs-on-chips. Trends Cell Biol 21:745–754. https://doi.org/10.1016/J.TCB.2011.09.005
138. Kunz-Schughart LA, Freyer JP, Hofstaedter F, Ebner R (2004) The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. J Biomol Screen 9:273–285. https://doi.org/10.1177/1087057104265040
139. Ho WJ, Pham EA, Kim JW et al (2010) Incorporation of multicellular spheroids into 3-D polymeric scaffolds provides an improved tumor model for screening anticancer drugs. Cancer Sci 101:2637–2643. https://doi.org/10.1111/J.1349-7006.2010.01723.X
140. Drewitz M, Helbling M, Fried N et al (2011) Towards automated production and drug sensitivity testing using scaffold-free spherical tumor microtissues. Biotechnol J 6:1488–1496. https://doi.org/10.1002/BIOT.201100290
141. Pampaloni F, Reynaud EG, Stelzer EHK (2007) The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 8:839–845. https://doi.org/10.1038/nrm2236
142. van Meer PJK, Kooijman M, Gispen-de Wied CC et al (2012) The ability of animal studies to detect serious post marketing adverse events is limited. Regul Toxicol Pharmacol 64:345–349. https://doi.org/10.1016/J.YRTPH.2012.09.002

143. Huh D, Torisawa YS, Hamilton GA et al (2012) Microengineered physiological biomimicry: organs-on-chips. Lab Chip 12:2156–2164. https://doi.org/10.1039/C2LC40089H
144. Zhang B, Radisic M (2017) Organ-on-a-chip devices advance to market. Lab Chip 17:2395–2420. https://doi.org/10.1039/C6LC01554A
145. Caplin JD, Granados NG, James MR et al (2015) Microfluidic organ-on-a-chip technology for advancement of drug development and toxicology. Adv Healthc Mater 4:1426–1450. https://doi.org/10.1002/ADHM.201500040
146. Bhagat AAS, Jothimuthu P, Papautsky I (2007) Photodefinable polydimethylsiloxane (PDMS) for rapid lab-on-a-chip prototyping. Lab Chip 7:1192–1197. https://doi.org/10.1039/B704946C
147. Knowlton S, Yenilmez B, Tasoglu S (2016) Towards single-step biofabrication of organs on a chip via 3D printing. Trends Biotechnol 34:685–688. https://doi.org/10.1016/J.TIBTECH.2016.06.005
148. Park JY, Jang J, Kang HW (2018) 3D bioprinting and its application to organ-on-a-chip. Microelectron Eng 200:1–11. https://doi.org/10.1016/J.MEE.2018.08.004
149. Amin R, Knowlton S, Hart A et al (2016) 3D-printed microfluidic devices. Biofabrication 8:1–16. https://doi.org/10.1088/1758-5090/8/2/022001
150. Kankala RK, Lu F-J, Liu C-G et al (2018) Effect of icariin on engineered 3D-printed porous scaffolds for cartilage repair. Materials 11:1–16. https://doi.org/10.3390/MA11081390
151. Yi Z, Kankala RK, Wang S-B, Chen A-Z (2019) Multi-organs-on-chips: towards long-term biomedical investigations. Molecules 24:1–22. https://doi.org/10.3390/MOLECULES24040675
152. Sweeney LM, Shuler ML, Babish JG, Ghanem A (1995) A cell culture analogue of rodent physiology: application to naphthalene toxicology. Toxicol In Vitro 9:307–316. https://doi.org/10.1016/0887-2333(95)00007-U
153. Sung JH, Dhiman A, Shuler ML (2009) A combined pharmacokinetic-pharmacodynamic (PK-PD) model for tumor growth in the rat with UFT administration. J Pharm Sci 98:1885–1904. https://doi.org/10.1002/JPS.21536
154. Sin A, Chin KC, Jamil MF et al (2004) The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors. Biotechnol Prog 20:338–345. https://doi.org/10.1021/BP034077D
155. Huh D, Fujioka H, Tung Y-C et al (2007) Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems. Proc Natl Acad Sci U S A 104:18886–18891. https://doi.org/10.1073/PNAS.0610868104
156. Schwartz MP, Hou Z, Propson NE et al (2015) Human pluripotent stem cell-derived neural constructs for predicting neural toxicity. Proc Natl Acad Sci U S A 112:12516–12521. https://doi.org/10.1073/PNAS.1516645112
157. Belair DG, Whisler JA, Valdez J et al (2015) Human vascular tissue models formed from human induced pluripotent stem cell derived endothelial cells. Stem Cell Rev Rep 11:511–525. https://doi.org/10.1007/S12015-014-9549-5
158. Sobrino A, Phan DTT, Datta R et al (2016) 3D microtumors in vitro supported by perfused vascular networks. Sci Rep 6:1–11. https://doi.org/10.1038/SREP31589
159. Hsu Y-H, Moya ML, Hughes CC et al (2013) A microfluidic platform for generating large-scale nearly identical human microphysiological vascularized tissue arrays. Lab Chip 13:2990–2998. https://doi.org/10.1039/C3LC50424G
160. Vunjak-Novakovic G, Bhatia S, Chen C, Hirschi K (2013) HeLiVa platform: integrated heart-liver-vascular systems for drug testing in human health and disease. Stem Cell Res Ther 4 (Suppl 1):S8. https://doi.org/10.1186/SCRT369
161. Zhu J, Xu Y, Rashedi AS et al (2016) Human fallopian tube epithelium co-culture with murine ovarian follicles reveals crosstalk in the reproductive cycle. Mol Hum Reprod 22:756–767. https://doi.org/10.1093/MOLEHR/GAW041

162. Shirure VS, George SC (2017) Design considerations to minimize the impact of drug absorption in polymer-based organ-on-a-chip platforms. Lab Chip 17:681–690. https://doi.org/10.1039/C6LC01401A

Futuristic Aspects of Human Organ on a Chip

11

Martin Dufva

Abstract

The futuristic human organ on a chip (HOOC) is going to replace experimental animals, but many basic functions need to be implemented to reach that goal. Today, it is possible to keep 10–13 connected organs alive for 1–3 weeks without losing a significant function in most of the organs in the system. This number of organs is sufficient to model a large part of the basic physiology of an organism. Selecting which cell models to use and how to scale these models relative to each other to best mimic an organism is still not obvious and will be the research for the next decade. However, the road to that is long and will involve engineering better hardware solutions, obtaining more knowledge about the interplay between organs and tissue, and developing better and more accessible cell models.

11.1 Introduction

Future human organ on chips systems (HOOCS) are likely going to be split into different complexity levels regarding the cell source and abilities to simulate human physiology. The correct ambition level needs to be chosen for a given application or biological problem. The most complex HOOCS today have 10–13 interconnected organs in a physiologically relevant fluidic network [1, 2]. The fluidics is driven by the complex machinery of actuators and computers, etc. More than 10 interconnected organ models are considered high-end and the aim for these systems is a true 1:1 replica of an experiential animal but with human cells. The throughput of these systems is usually low, and costs are high due to the complex machinery needed to

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drive these systems. These high-end microphysiology systems are excellent for answering important questions such as how a drug is administrated, distributed, metabolised and excreted (ADME) [3, 4], which requires many interconnected organs but not necessarily all. Hence, the complexity can be reduced to 4 or 5 organs relevant for the ADME and the disease models. In other cases, a single organ on a chip can be useful for modeling a disease. HOOCS are complementing experimental animals and ultimately humans subjects to describe what is happening on the system level. The motivation for HOOCS is often to replace experimental animals for drug and toxicity testing. However, reverse engineering a living organism using HOOCS systems will provide a rich source of information on how the human body works. The ability to add and remove an organ will provide a unique opportunity to study how organs interact with each other via paracrine factors. The latter work has just begun as we currently do not understand all signaling happening in the body.

In many cases in biology research, it is advantageous to study the separate parts individually and later gather system-level information. This progression in complexity is often seen in biology research where simple cell models are used to understand the mechanisms and a knockout mouse is used to describe the effect on the system level. This approach is efficient and has laid the foundation for the current knowledge of molecular and cell biology. Mechanistic research on the gene or single cell type level might put less demand on modeling the correct physiology but demands high throughput and therefore ease of use. Alternatives such batch culture systems are likely competitive in this context but may in the future be complemented with single organ on a chip model if these have significantly better functions and especially if these are as simple to use [5].

11.2 Platforms

11.2.1 Wells and Flasks

Much effort has been put into making excellent batch models of various tissues by optimising extracellular matrix protein (ECM) compositions, hydrogels to obtain correct softness and 3D environment, and mediums. Much of this research is performed using plates and flasks and is directly competing with HOOC for obtaining cells with high functionality. It is noteworthy that nearly all cell culture protocols have first been developed for batch culture before being applied on the chip. Similarly, nearly all cell lines and natural isolates have been isolated and cultured firstly on hard polystyrene, possibly with a coating of hydrogel or an extracellular matrix. This means that cells have been selected or adapted for rapid growth on hard or semihard surfaces during batch culture. In most cases, very little optimisation of culture and differentiation protocols has been done on chip even though factors are typically at much higher local concentration during perfusion. The mechanism behind the improved functions observed with fluidics is usually not satisfactorily explained in the literature. The research community has many opportunities here to optimise the cell culture conditions and cell sources for the

flow environment. This optimisation may also unravel positive and negative autocrine and paracrine loops [6] as well as demands for nutrients and waste removal [7, 8].

The traditional cell culture system (well, flasks, Transwell) is highly modular, easy to use and commercially available. The well is simple to use because it is operated with a pipette after unscrewing a cap or lifting a lid. The standardised format is furthermore essential for use with external instruments, such as TEER (trans epithelial/endothelial electrical resistance) measuring devices, microscopes and plate readers. The format is also compatible with all sorts of many-step staining protocols, 'eomics', and other analysis techniques. This makes the infrastructure of the traditional cell culture system very cost-effective as the cultures are portable between instruments. Adding the possibility to connect different organs, mixing and shear by, for instance, orbital shaking would make this a compelling platform for high content screening and mechanistic research.

Advanced well plates batch cultures with many connected cell types, patterning of hydrogels and simpler shear systems are the first step from 2D static culture (see Fig. 11.1 for a comparison between batch and fluids). This first step is achieved using Transwell plates where multi-organs or tissues are cultured together often with improved functions [9]. Much of the research about barriers has been done with Transwell systems rather than using HOOCS because the latter is more difficult to work with or is not commercially available. Shear on endothelial and epithelial cells can be obtained with rocker systems or orbital shakers. In fact, an orbital shaker can achieve >10 dyn/cm^2 at the periphery of a well [10, 11], which is competitive with shear obtained in fluidic systems. Such a low-tech solution also mixes the liquid rapidly to ensure that concentration gradients of, for example, nutrients are as steep as possible just as in microfluidics systems.

These simple actuation systems have also been utilised in the microfluidics community to introduce shear and mixing in a user-friendly way [12]. It is expected that further improvements in batch culture hardware will increase its abilities to model physiology. Adding shear and mixing and means to interconnect organ models is the first step to very advanced 'Organ systems in a well' [10, 11, 13] (Fig. 11.2a).

Robotic automation of batch culture would theoretically allow for frequent mediums shifts, temporal changes in mediums compositions and frequent sampling for analysis. This is an underrepresented approach for coupling organ models together and to model physiological events. One of the main roadblocks with such dedicated robotics is that the whole instrument needs to be put into an incubator or the whole incubator needs to work as a CO_2 incubator with humidity control [14].

11.2.2 Microfluidics

The next complexity level is 'organ' or 'cell type' on a chip (Fig. 11.2b) and these chips are excellent to verify mechanisms on the gene, cell type, organ level as these may provide much more relevant data than a simpler batch model [15, 16]. The

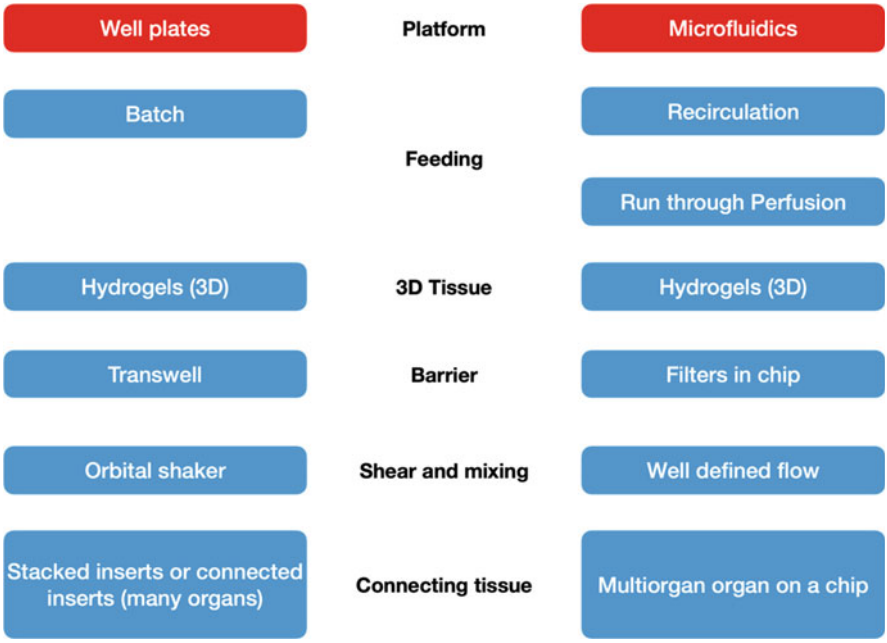

Fig. 11.1 There are two basic platforms—well plates (batch) and microfluidics. Many of the functions needed for obtaining shear, barriers, 3D tissue environment and means to interconnect different tissue models have been implemented in the respective platforms. Mostly, the main advantage of microfluidics is its precision of flow, while the corresponding features in well plates are less precise

hardware is defined as chips, inserts, pumps, valves and sensors. Each of these components is high-performing of well known technology. The difficulties begin when many of these chips will be combined into one easy-to-use and affordable system that also is compatible with analysis instruments and sterile workflows. As indicated in Fig. 11.2b, a multi-organ system requires pumps and/or valves and means to interconnect each organ into a system. The complexity increases rapidly with each added organ model.

11.2.3 Insert/Microfluidic Hybrids

In plate/microfluidic hybrids, organ models are added to a fluidic network as individual inserts (like standard Transwell inserts) instead of chips (compare Fig. 11.2b, c). The fluidic base is either driven by external peristaltic pumps or has PDMS valves working as pumps integrated into the base. As each subunit works as a removable insert, it is easy to disconnect and mount the inserts into instruments for analysis. This approach has enabled 10–13 organ models to be connected [1] and

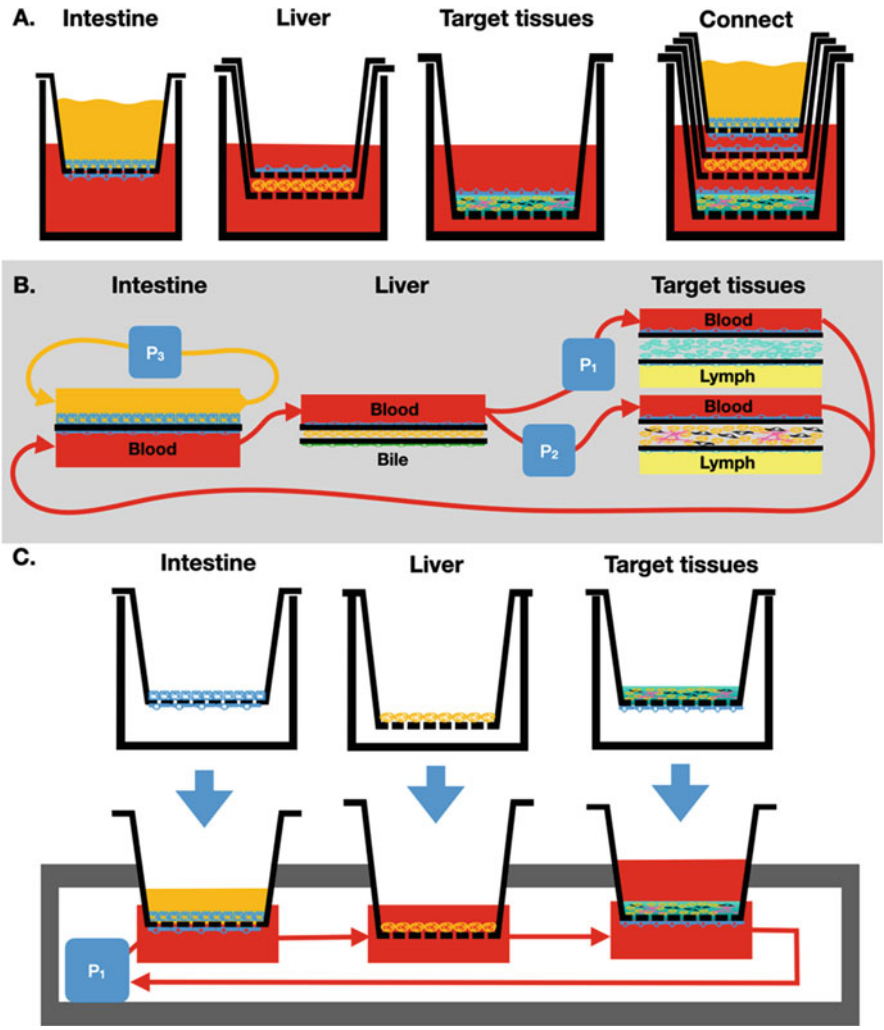

Fig. 11.2 Different platforms for interconnecting organs. (**a**) 3D printed inserts can be stacked on top of each other forming an organ system in a well. This is a simple approach to interconnect organs, but the only mixing and shear that can be obtained is via an orbital shaker or similar actuators. However, the freedom of 3D printing would allow for many implementations of these simple stacking systems to improve, for instance, interorgan communication via gravity flow. (**b**) Traditional microfluidics where the liquid forms a closed loop. The respective organ models (each chip) are typically very well defined and functioning but, as apparent, are difficult to interconnect and therefore to operate. (**c**) Hybrid between the well and fluidics where each organ system is attached to a base containing all liquid handling and fluidics

commercial versions exist (CN-Bio PhysioMimix system). This approach is in stark contrast to traditional fluidic chips that are part of a closed fluidic loop [15]. The partly open system enables easy access to culture and an easy way to plug in new

organs without having risks with, for instance, bubbles. By contrast, the traditional chips in closed fluidic paths systems always have difficulties with changing medium (bubble formation), and therefore bubble traps are incorporated. Bubble traps are generally not a good approach as these often increase the dead volume of the system. The closed fluidic path also relies on being connected to a pump system, which makes some of them immobile. Mobility is required for analysing cells in situ in for instance microscopes. A future system would ideally have all the pumping capacity on a small device that, when connected to electricity, drives a complete HOOCS. Such a device needs to be self-sustained and the medium should be changed by pipetting into a well. Small footprint peristaltic pumps would be able to drive a complete HOOC and still be quite compact so that many of these devices can be put into an incubator on top of each other.

11.2.4 Gravity-Driven Flow

Chips driven by gravity are a very interesting alternative for some applications. These chips have typically a fluidic unit consisting of two wells and a channel that connects these two wells. The liquid is moved between the two wells by periodically rocking the unit. Mimetas has commercialised this where the most advanced system has three connecter subunits. The middle unit is for modifying the chips with a hydrogel ('tissue'), while the two other units model, for instance, the intestine and the blood [5]. The Mimetas plate/chips, therefore, model most of the tissues in the body. Mimetas has 40 of these operating in parallel in a standard 384-well plate format, which makes it usable for high content screening. There are possibilities to connect two or more organs by gravitational flow and to obtain unidirectional flow [12]. However, the drawback is that to obtain consistent flow, the respective channels need to be micromachined with high precision, which is costly. Furthermore, the range of flows obtained with one chip is limited as the flow velocity is determined by the rocking angle and channel dimensions. It may therefore be difficult to model both high and low flow velocities on one chip.

11.3 Sensors

There are destructive end-point sensings such as transcriptome analysis that can be applied to both batch and chip systems and these will not be discussed more in this chapter. In batch systems, all sensors are 'offline' meaning that the culture device is passive and does not contain any integrated sensors. Sensing is done by placing the cell culture device into instruments such as microscopes and plate readers to assess cell morphology and the reporter signals (bioluminescence and fluorescence) indicating the presence of a biomarker or an activity. This is one example of non-invasive assays that can be done on live cells. Another non-invasive assay is to use electricity (trans epithelial/endothelial electrical resistance (TEER)) to assess barrier function. Non-invasive methods based on temporarily adding sensors to the

plate allow for time-lapse studies. This is a cost-effective approach that typically gives infrequent but often sufficient sampling but requires human interactions. For proper online monitoring, the sensors and reading devices can be integrated into the chip. This increases the complexity of the chip but gives opportunities to follow rapid changes as they happen. Interfacing chips with expensive high-end instruments such as microscopes and mass spectrometers are possible but seldom used as the cell culture may take days or weeks and the instruments are not usable for others in that period. Some smaller and relatively cheap microscopes are possible to integrate with a chip system of various kinds. Some of these instruments can also be put into an incubator.

11.4 What Has Not Been Achieved in Any Platform Yet in Terms of Physiology?

Recent advances in HOOC research have demonstrated high-performing models that often display certain advantages compared to corresponding batch cultures. Many of these systems are said to be microphysiological systems because they, as accurately as possible, mimic a subset of human physiology. As outlined below, some very basic physiological functions are not established today in HOOCS. Running multi-HOOC systems with accurate physiology mimicking is therefore still in its infancy.

11.4.1 Fluctuating Nutrients and Hormone Profiles

In a human, there is a daily cycle of hormones that are released and degraded (Fig. 11.3). There are many hormones that regulate for instance metabolism and activity on the body, and these are typically not modeled in HOOCs. For instance, the diurnal cortisol levels have a large impact at the organism level and have been implied in many diseases including diabetes, cardiovascular disease and depression [17]. Similarly, thyroid hormones (T_3 and T_4), which have a large impact on metabolism and development, are controlled by hormones released in the pituitary (thyroid-stimulating hormone), which is in turn regulated by the hypothalamus via the release of thyrotropin-releasing hormone [18]. Futuristic chips may include these and other central hormone systems to mimic the daily rhythm of humans.

Humans eat about three to four times a day, meaning that the availability of sugar and other nutrients is fluctuating. Blood sugar level fluctuates between 3.5 and 6.9 mM. In batch cell culture, the nutrient and sugar levels are also fluctuating but on a typical 2–3-day basis. Cell culture medium has 5–11 mM sugars to provide a store of sugar that is sufficient to feed the cells 2-3 times a week. In the rich mediums, cells are overfed on day 1 and possibly starving on days 2 or 3. Simulating, three meals a day would be possible to achieve in HOOCS as these are highly automated and programming of a feeding pattern is a simple task but increases the complexity of the system as it requires a pump line for a null medium and an individual pump line for each individual factor being controlled [19]. The

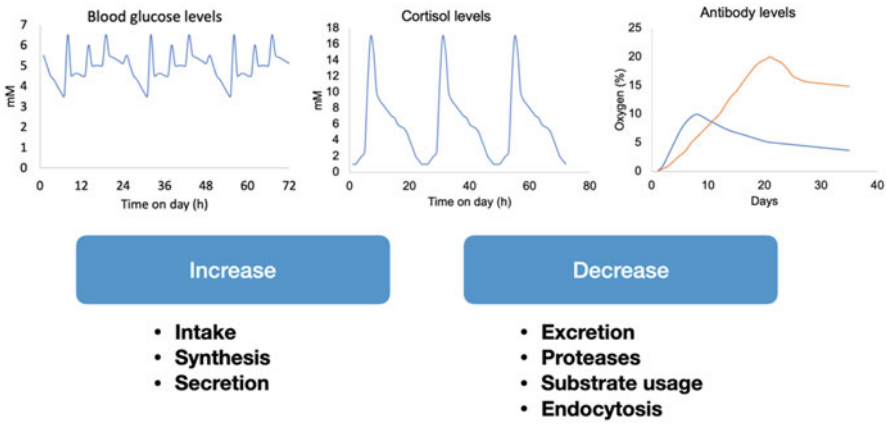

Fig. 11.3 Diurnal rhymes and other transient phenomena. Nearly all factors change their levels in plasma during the day or over weeks and sometimes years. Some factors such as salt and pH balance are very tightly controlled and similarly O_2 pressure is nearly constant over a day. By contrast some hormones such as cortisol changes levels daily. Blood sugar and correspondingly insulin also change over a day but are more frequent than the cortisol level. Antibodies are secreted and degraded over weeks. In general, there are mechanisms that add (usable factors), such as intake (food) synthesis and secretion, and decrease the concentration of factors. The increase and decrease mechanisms are part of a regulatory network resulting in homeostasis or reaction to external cues

precise dosage of nutrients and hormones can be controlled individually to obtain the correct profile over a day. However, this is as said complex, and it is therefore seldom seen implemented in HOOC systems. Instead, the medium is either recirculated through HOOCS and changed every other day or a fresh medium is constantly pumped through the HOOC model. Any type of recirculation within frequent medium changes is conceptually very similar to batch culture as the nutrients are declining and waste products are increasing over time.

The accumulation of waste and secreted signaling factors is also problematic to mimic. In the human body, waste is broken down and excreted through the intestine (via the liver) or through the urine via the kidneys. These functions are often not simulated well in HOOC systems due to a lack of appropriate cells models, or it is deemed not interesting for the aim of a specific HOOCS. Instead, the purification of the medium is achieved when switching the medium. Switching the medium three times a day would keep the toxic waste levels low but will also dilute secreted factors. In the human body, secreted factors such as antibodies and other proteins are filtered in the kidney to retain them. A dialysis approach to switch medium would be better to retain secreted proteins. However, the half-life of secreted factors in the blood is relatively short due to proteases and other mechanisms. In the absence of degradation mechanisms, factors will increase in concentration over time in any cell culture. When the cell medium is changed, these factors are effectively diluted but during a 2-day culture, the concentration can reach 100 nM [20] and thus in a range that affects cells.

To sum up, basic tasks of feeding, hormone cycles and disposal of waste and normal turnover of secreted factors should be modulated on chip on futuristic HOOCS. Numerous diseases are caused or modulated by these diurnal rhythms and turnover of factors. Modeling such diseases without these basic functions will be less relevant and affect the predictive power of HOOCs.

11.4.2 Vascularisation, Blood and Oxygen Pressure

The majority of HOOCS are modeling the blood vessel/tissue with a straight channel in Dimethylpolysiloxane (PDMS) with an approximate dimension of 100–500 μm in height, and length and width in the lower millimetre range. This pragmatic solution is far from the situation in the tissue but will mimic the cross section of a capillary, barrier, stroma unit sufficiently well. The blood and blood vessels have, however, numerous functions. One is to transport oxygen deep into the tissue. The intricate network of capillaries where the maximum distance is 200 μm between two capillaries ensures that all cells get enough O_2. The capillary network has however been very difficult to reproduce on chips as these typically need to be obtained in hydrogels. The fabrication of thin channels in hydrogels is difficult. Many techniques such as 3D printing have relatively poor resolution and the hydrogels are often too soft, and so capillaries collapse after fabrication. One option would be to use 3D printing technology for the larger vessels and then rely on neovascularisation to obtain the capillary network. A better 3D representation of the capillaries is desirable for future HOOCS.

The O_2 pressure in tissue is much lower in the body than under standard in vitro culturing conditions. If anything, the often-used PDMS chips are ensuring the high availability of O_2 as the surface-to-volume ratio is very high in chips. High oxygen pressure can have large effects on cells. For instance, stem cells keep their undifferentiated state better under low oxygen concentrations, hepatocytes along the acinus display different metabolic activities due to the decreasing O_2 pressure along the capillary toward the vein, and muscles produce lactic acid at low O_2 pressure and so on. Furthermore, high O_2 pressure leads to a higher level of reactive oxygen species, and therefore higher mutation frequencies and corresponding cell physiology defences are upregulated. The stem cell niches are thought to be residing inside the tissue between to capillaries. It is expected that stem cells are exposed to 1–8% O_2 (where 20% is maximum). Modeling and ensuring the correct O_2 pressure in tissue is therefore important. This is, however, difficult to do by engineering unless the O_2 is regulated by the cells themselves just like in the body. Active cells in a sufficiently deep hydrogel can mimic gradual oxygen depletion in tissue to some extent [21]. The reason is that cells close to channels use up O_2 and therefore grow more rapidly than cells deeper into the hydrogel block. This is also apparent in humans as the blood oxygen level is very low in the veins, which suggests that all O_2 has been taken up by the tissue and by laws of diffusion and competition for oxygen in metabolism, the cell closest to the blood has higher access to oxygen than the cell deeper into the tissue.

A key component of blood is the red blood cells. The main function is to transport oxygen from the lung and CO_2 from the tissue to the lung. The red blood cells as well as perfusion ensure that the whole body gets the correct amount of O_2. In the majority of HOOC applications, O_2 transport is reliable only on dissolved O_2 instead of being carried by the red blood cells. This may impact how long channels can be as the minute levels of dissolved O_2 rapidly are used up at the beginning of a long channel. Red blood cells are by contrast developed to release O_2 slower and in response to the surrounding O_2 levels. Therefore, a more even O_2 distribution in the artificial tissue can be expected. A futuristic HOOC system may include artificial red blood cells to achieve better control over the O_2 levels in the HOOC and to better mimic human physiology. This will require that the PDMS is replaced with materials that are oxygen impermeable, which is a huge change in fabrication techniques.

11.4.3 Immune System

Very few organ on a chip solutions can host an immune system reflecting its complete heterogeneity. Both T-cell and B-cell repertoires have a complexity of 10^8 clones, which are selected from a much larger initial repertoire. Along with these two adaptive immune systems, there are also the innate immune systems that are for instance responsible for inflammation and allergy. The innate immune systems consist of a handful of cell types that could be modelled on a chip by just adding them to the channel network or in a hydrogel to model for instance inflammation and atherosclerosis [22]. Similarly, reactive T cells and B cells can be added to a chip to delineate mechanisms of immune reactions. However, it is much more complicated to model the reaction towards for instance a virus infection as it means that the correct B-cell clone needs to be activated from a repertoire. HOOCS are not designed to handle 10^8 cells or more needed to model a complete repertoire. A preselected repertoire with less complexity ($10^{4–5}$ clones) might be possible to model on chip and it will still model recruitment of specific reactive clones.

In conclusion, futuristic HOOC will need to seamlessly include these examples and many more physiological systems to be competitive with the experimental animals.

11.5 Cell Sources and Modeling Healthy and Diseased Individuals

Currently, there is a mix of cell types in HOOC with very little standardisation. Cells can be cancer cell lines, primary cells and differentiated mesenchymal or pluripotent stems cells. That mix of cells is chosen because of availability, costs, ethics, function, or any combination of these. In the futuristic HOOCS, we will see donor-specific cells in all organs used in one system. The reason is that the genetic background is the same for all cells and therefore will provide donor-specific responses to a cue. In the future, patient-specific tissue will therefore be integrated

into the HOOC to mimic certain diseases. Many (not all) monogenetic diseases are likely easy to model on chip if there is a strong correlation between a genotype and a phenotype. In these cases, the human induced pluripotent stem cell is a good source to build patient-specific HOOCs with very few ethical problems. A polygenetic disease, that furthermore is triggered by the environment (for instance, atherosclerosis and diabetes), might be more difficult to make excellent models of that give information about the respective patient and population responses to a treatment.

With an appropriate differentiation protocol, it would be possible to re-create a complete HOOCS where all the cells come from one patient. The drivers for 10–13 organ systems exist but populating them with the different organs is however time-consuming and expensive as 10–13 different differentiation protocols need to be employed in parallel. Furthermore, the quality control in terms of measuring the activity of the respective organ needs to be assessed before connecting organs together to normalise the respective organ activities. It would not do that a micro liver had too high activity compared to the uptake rate from the intestine. It would be like modeling a human with a too large liver, and conversely the rate of for instance drug breakdown will be overestimated. Therefore, it is not expected in the foreseeable future that respective organs in a HOOC can be developed together due to medium incompatibilities and scaling issues. The respective organs must therefore be developed independently from each other and subsequently be connected at an appropriate developmental stage. Much more research is needed to optimise organ model development underflow and know when to connect organ models so they can codevelop into an artificial organism.

A simpler middle way may be to have a 'standard' HOOC model where only the tissue/organ that has the diseases will be modelled with the patient's induced pluripotent stem cells. It would therefore be possible to simplify the production of the cell models on the HOOC. Standardisation may decrease costs and improve reproducibility, but there is very little knowledge if such a standard model will be associated with limitations in the predictive value for a population. Already today, it is a common practice to test a differentiation protocol on different donors to avoid bias. It is also well recognized that the inbreeding of animals needed to reduce variability has an impact on the predictive value. With that perspective, it may be better to re-create all organs on the HOOC using patient-specific cells.

Currently, HOOC can, at best, replace some animal trials focused on understanding for instance the efficacy of a drug or interactions between organs. For such focused research, HOOCS with one to a few connected organs will be sufficient. The ambition can however be much higher and include testing drug efficacy on the population level before going into a clinical trial. This might be done using focused HOOCS, but a larger systemic HOOC might be more informative as it also gives more information about toxicity and predicted side effects. Irrespective of the ambition level, these types of questions require that hundreds of patients' specific HOOCS are tested. This puts large demands on the hardware solution (see above) and automated differentiation of the various cell types, probably employing robotics and standardised batch cultures to obtain the correct cell types.

References

1. Edington CD, Chen WLK, Geishecker E et al (2018) Interconnected microphysiological systems for quantitative biology and pharmacology studies. Sci Rep 8:1–18. https://doi.org/10.1038/s41598-018-22749-0
2. Maschmeyer I, Lorenz AK, Schimek K et al (2015) A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. Lab Chip 15:2688–2699
3. Ishida S (2018) Organs-on-a-chip: current applications and consideration points for in vitro ADME-Tox studies. Drug Metab Pharmacokinet 33:49–54
4. Shinha K, Nihei W, Ono T, Nakazato R, Kimura H (2020) A pharmacokinetic-pharmacodynamic model based on multi-organ-on-a-chip for drug-drug interaction studies. Biomicrofluidics. https://doi.org/10.1063/5.0011545
5. Trietsch SJ, Israëls GD, Joore J, Hankemeier T, Vulto P (2013) Microfluidic titer plate for stratified 3D cell culture. Lab Chip 13:3548–3554
6. Hemmingsen M, Skafte-Pedersen P, Sabourin D, Andersen RF, Sørensen AL, Collas P, Dufva M (2011) Perfusion cell culture reveals a paracrine or autocrine signalling pathway involved in adipose-derived stem cell differentiation into adipocytes. In: 15th International conference on miniaturized systems for chemistry and life sciences 2011, MicroTAS 2011, vol 2
7. Guzzardi MA, Domenici C, Ahluwalia A (2011) Metabolic control through hepatocyte and adipose tissue cross-talk in a multicompartmental modular bioreactor. Tissue Eng Part A 17:1635–1642
8. Guzzardi MA, Vozzi F, Ahluwalia AD (2009) Study of the crosstalk between hepatocytes and endothelial cells using a novel multicompartmental bioreactor: a comparison between connected cultures and cocultures. Tissue Eng Part A 15:3635–3644
9. Hatherell K, Couraud PO, Romero IA, Weksler B, Pilkington GJ (2011) Development of a three-dimensional, all-human in vitro model of the blood-brain barrier using mono-, co-, and tri-cultivation Transwell models. J Neurosci Methods 199:223–229
10. Driessen R, Zhao F, Hofmann S, Bouten C, Sahlgren C, Stassen O (2020) Computational characterization of the dish-in-a-dish, a high yield culture platform for endothelial shear stress studies on the orbital shaker. Micromachines. https://doi.org/10.3390/MI11060552
11. Dardik A, Chen L, Frattini J, Asada H, Aziz F, Kudo FA, Sumpio BE (2005) Differential effects of orbital and laminar shear stress on endothelial cells. J Vasc Surg. https://doi.org/10.1016/j.jvs.2005.01.020
12. Wang YI, Shuler ML (2018) UniChip enables long-term recirculating unidirectional perfusion with gravity-driven flow for microphysiological systems. Lab Chip. https://doi.org/10.1039/c8lc00394g
13. Leth Jepsen M, Willumsen A, Mazzoni C, Boisen A, Hagner Nielsen L, Dufva M (2020) 3D printed stackable titer plate inserts supporting three interconnected tissue models for drug transport studies. Adv Biosyst. https://doi.org/10.1002/adbi.201900289
14. Novak R, Ingram M, Marquez S et al (2020) Robotic fluidic coupling and interrogation of multiple vascularized organ chips. Nat Biomed Eng 4:407–420
15. Ingber DE (2020) Is it time for reviewer 3 to request human organ chip experiments instead of animal validation studies? Adv Sci 7:1–15
16. Herland A, van der Meer AD, FitzGerald EA, Park T-E, Sleeboom JJF, Ingber DE (2016) Distinct contributions of astrocytes and pericytes to neuroinflammation identified in a 3D human blood-brain barrier on a chip. PLoS One 11:e0150360
17. Adam EK, Quinn ME, Tavernier R, McQuillan MT, Dahlke KA, Gilbert KE (2017) Diurnal cortisol slopes and mental and physical health outcomes: a systematic review and meta-analysis. Psychoneuroendocrinology 83:25–41
18. Philippe J, Dibner C (2015) Thyroid circadian timing: roles in physiology and thyroid malignancies. J Biol Rhythms 30:76–83

19. Skafte-Pedersen P, Hemmingsen M, Sabourin D, Blaga FS, Bruus H, Dufva M (2012) A self-contained, programmable microfluidic cell culture system with real-time microscopy access. Biomed Microdevices 14:385–399
20. Hemmingsen M, Vedel S, Skafte-Pedersen P, Sabourin D, Collas P, Bruus H, Dufva M (2013) The role of paracrine and autocrine signaling in the early phase of adipogenic differentiation of adipose-derived stem cells. PLoS One. https://doi.org/10.1371/journal.pone.0063638
21. Pimentel R, Ko SK, Caviglia C et al (2018) Three-dimensional fabrication of thick and densely populated soft constructs with complex and actively perfused channel network. Acta Biomater 65:174–184
22. Polini A, del Mercato LL, Barra A, Zhang YS, Calabi F, Gigli G (2019) Towards the development of human immune-system-on-a-chip platforms. Drug Discov Today 24:517–525

Development of Human-on-a-Chip

12

Eden Mariam Jacob, Ankita Borah, and D. Sakthi Kumar

Abstract

Traditional 2D (two-dimensional) in vitro cultures cannot replicate the in vivo physiological environment. Although animal models have been widely used in the preclinical testing of drugs, their inability to replicate the accuracy in pharmacokinetic and pharmacodynamic profiles of the human physiology owing to interspecies dissimilarity often succumb to failure in the clinical trial phase. The paucity of therapeutic efficacy and their severe side effects are some of the additional reasons for drug failure, which are undetected in the current in vitro and in vivo models. Considering the development of new drugs, cost and time are two important parameters, where only a few get approved eventually by the authorities. All these plausible reasons propelled scientists to come up with more physiologically relevant representative human organ models that will be worthwhile in preclinical drug testing. Multiorgan microphysiological (MOM) systems are the new-age miniature in vitro human biomimetics that can mimic the intra/interorgan interactions to predict the drug responses accurately. These systems could be used for the evaluation of drug toxicity and drug metabolism in a single device, thus providing a platform to conduct translational and precision medicine research with ease and circumvent the challenges associated with the traditional in vitro and animal models. In this chapter, we discuss the various

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types and techniques of MOM systems, their applications, and their advantages/limitations.

12.1 Introduction

The drug development process over the years in pharmaceutical companies endures a long arduous journey to bring a clinically effective and safe drug, from bench to bedside. In this extensive process of drug discovery, one of the crucial steps that highly affect the success of the drug in clinical trials is preclinical drug screening. The traditional method of conducting preclinical drug screening uses in vitro cell culture systems and reliable animal models to study the predictability and efficacy of the concerned drug in humans. One of the widely used in vitro cell culture techniques is the 2D cell culture system, where cells are grown on a two-dimensional plastic surface under a controlled environment. These systems are easy to use and maintain, widely available, and involve low costs that somehow assist in the rapid high-throughput screening process of drug discovery. Nonetheless, with such benefits 2D cell culture systems fail to emulate the human physiological environment, primarily the complex cell/tissue-matrix interactions, inter/intra organ interactions, and immune cell interactions that contribute to the failure of a large percentage of drugs in the preclinical stage to progress into the successful clinical translation phase. Henceforth, the use of animal models comes to the rescue in this scenario, to say the least, and can recapitulate the living system to some extent. Adding to the disappointment, animal models still differ from the human genome, physiology, and anatomy contributing to varied drug responses and false data in terms of human pathophysiology finally succumbing to attrition. Furthermore, animal models raise ethical issues, and it is an expensive and time-consuming process. To date, only 10–15% of the drugs are clinically approved for human use following their successful preclinical drug testing in animal models [1–4]. Consequently, there is an urgency regarding the need to innovate and develop state-of-the-art in vitro systems that will recapitulate the complexities of the human organs and the critical features that overcome the caveats of 2D cell culture systems and existing in vivo models.

12.2 Why Do We Need a Human-on-a-Chip System?

Imagine a miniature version of the human body that can mimic its entire biological functions and can be used in the pharmaceutical companies to analyze the different drug responses during the preclinical drug testing process. This idea of developing miniaturized copies of your body started in the early 2000s, which were called the microcell culture analogs (CCAs) comprising living cells grown on a surface or embedded within a 3D hydrogel matrix connected by channels measured in hundreds of micrometers that represents blood flow [5]. This device was fabricated

to mimic the multiorgan interactions where cells representing the liver (HepG2/C3A), tumor (HCT-116), and bone marrow (Kasumi-1) were grown in separate chambers and was used to analyze the pharmacokinetic and pharmacodynamic profiles of an anticancer oral prodrug Tegafur. On comparison with the experimental results obtained from the 2D culture-based 96-well plate analysis, it was revealed that the micro CCA system metabolized Tegafur to its active component 5-fluorouracil in the liver compartment leading to cell death while the cells in the 96-well plate failed to do so, thus proving the physiological relevance of using this microsystem in drug testing [5]. This state-of-the-art technology to develop mini versions of the human body and its organs is more generally referred to as microphysiological systems (MPS). Microphysiological systems over the past decade have seen staggering advancements in the development of different versions, including organoids, 3D-bioprinted tissue constructs, and organ-on-chip models, and are receiving due attention from the scientific community to explore their potential biomedical applications owing to their ability to mimic closely the human organ-like features in an in vitro setting. Now comes the question as to why these systems are required in the current scenario of existing animal models that are already being used in the drug discovery process. The first answer is that MPS lacks neuronal and mental complexities and capacities that will not give rise to any pain and suffering unlike caused to the sentient beings. Adding to that, the use of animal models is a time, research-intensive process and bears expensive costs. Furthermore, another factor is the discrepancies in the drug response and the pathophysiology.

These microphysiological systems are seen as "human surrogates" that should have the characteristics to recapitulate the drug pharmacodynamics of absorption, distribution, metabolism, and excretion (ADME), drug pharmacokinetics, and finally project as an improved and accurate predictive model in the drug screening process that will lead to the successful clinical translation of drugs reducing their costs and attrition rates [6]. One of the promising technologies to construct such human surrogates is the physiological-based pharmacokinetic (PBPK) mathematical modeling providing a mechanistic approach to investigate and predict the pharmacokinetics (PK) of a given drug [7]. This is a bottom-up approach where the human body is considered an interconnected series of compartments, for example, reactors and absorbers, and each compartment is characterized as a single homogeneous entity of organs or tissues, thus reflecting a scenario of organ-organ interactions. Different concentrations of drug and their metabolites in the compartments are described using differential equations and it can be useful to predict the PK of a given drug, drug dose, comparative analysis of the drug between normal and diseased states when combined with pharmacodynamic (PD) tools [7]. The ultimate goal of therapeutic intervention is to treat a patient without causing any unnecessary severities and the use of such PBPK modeling tools would help to achieve myriad opportunities to improve drug development and lead them to their successful clinical applications. An early prototype of the PBPK model was constructed having lung, fat, kidney, liver, and other tissues as the compartments to study the uptake and metabolism of toxicant naphthalene by rodents and mice and predicted the

toxicological response of naphthalene based on the dose, pretreatments, and routes of administration when tested in animals [8]. Contrary to the PBPK model, the cell culture analog (CCA) system mimics the living system realistically in terms of organ-organ interactions, thus assisting in in vitro drug testing more accurately. The construction of the CCA system is inspired by the PBPK modeling approach where it allows to monitor the biotransformation of drugs, metabolite exchange between the organs, and similar drug doses could be used in animal tests that help in extrapolation to humans. A proof-of-concept of the CCA system was developed by Shuler et al. where they compartmentalized different cells of interest such as hepatocytes for the liver in multiple compartments and bioreactors and analyzed the metabolism of the test compound naphthalene [9]. It was revealed that the interconnected cell compartments provided with continuous cell medium led to the circulation of the cytotoxic naphthalene metabolites produced by the liver hepatocyte cells. These metabolites circulated throughout the chambers and led to the killing of the lung cells in the lung chamber. This kind of CCA model is beneficial to assess the remote activities of the drug compounds and their metabolites produced in one organ/tissue cross-talk with other tissues, lacking in traditional cell culture models [9].

Organ-on-a-chip mimics the microenvironment of organs/tissues built on an artificial microstructural microfluidic chip platform incorporating cell biology, medical science, biotechnology, and microfabrication concepts [10]. Organ-on-a-chip on its own is a sophisticated model to study the detailed organ physiology and assessment of the drug responses and their underlying mechanisms. Nevertheless, no organ works in a solitary manner and is in constant communication with one another to maintain homeostasis [11]. This interorgan interaction and communication to emulate the human physiological condition is represented by another multiorgan microphysiological (MOM) system that has gained momentum in recent years due to the rapid technological advancements in microfluidics and micro/nanofabrication techniques and is the engineering of human cell-based microscale multiorgan models also popularly known as body-on-a-chip (BOC) systems [12, 13] (Fig. 12.1). BOCs are fabricated to recapitulate human physiology by integrating all the major organ systems, the size ratio of the organs, maintaining medium flow rate, and physiological fluid-to-tissue ratios to study the complex PK and PD of compounds and tissue-organ interactions [11], which will assist in the extrapolation of the data from these systems to humans with improved accuracy levels. Hence, the development and application of BOCs in drug discovery and other biomedical applications is an absolute necessity in today's time.

12.3 Types of Multiorgan Integration Platforms

Simulation of dynamic interorgan interactions in a single system with the help of microfluidic technologies is a prominent feature of body-on-a-chip (BOC) systems. BOC is divided into four major categories based on their interconnected fluidic configurations: (1) static microscale platforms, (2) single-pass microfluidic

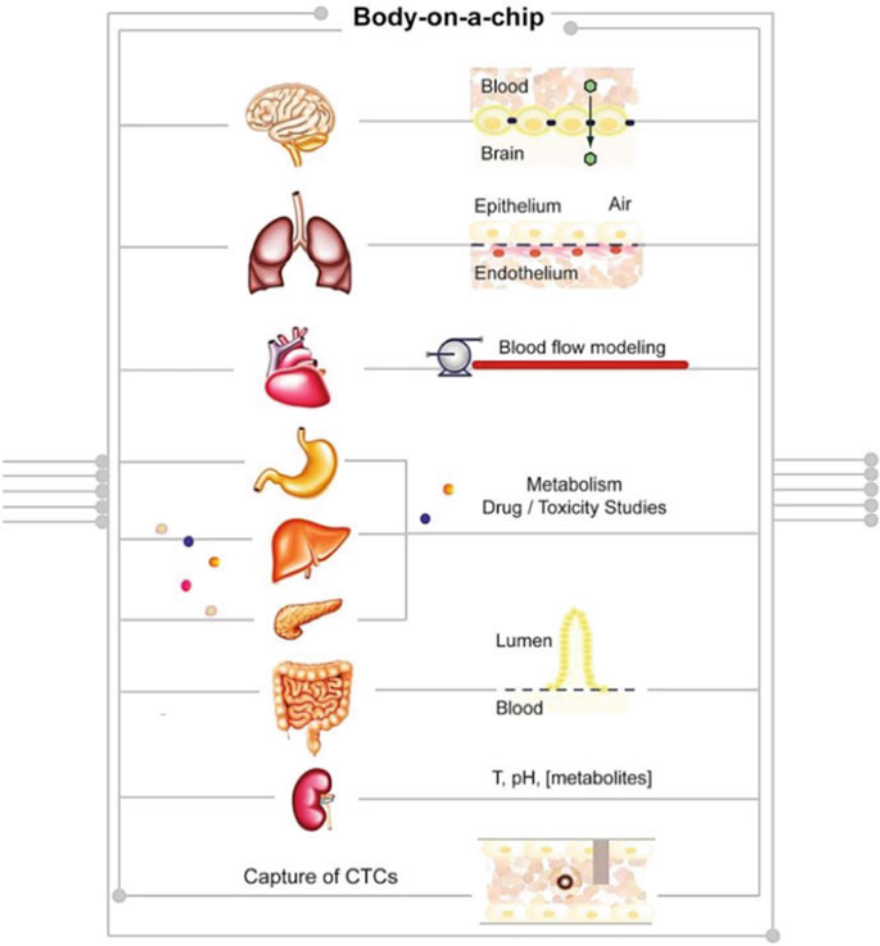

Fig. 12.1 Schematic representation of a BOC approach in which cell-autonomous and non-autonomous studies can be performed using a single chip. (Adapted and Copyright from Perestrelo, A. R., Águas, A. C., Rainer, A., & Forte, G. (2015). Microfluidic Organ/Body-on-a-Chip Devices at the Convergence of Biology and Microengineering. Sensors (Basel, Switzerland), 15(12), 31142–31170. https://doi.org/10.3390/s151229848 [14]. Under Creative Commons Attribution License 4.0)

platforms, (3) pump-driven recirculating microfluidic platforms, and (4) pumpless recirculating microfluidic platforms [6].

12.3.1 Static Microscale Platforms

In static microscale platforms, the different organ compartments achieve interorgan communication via direct physical contact through a common medium. This model

ensures the passive diffusion of soluble factors, ligands, cellular metabolites, and components across the interconnected organ compartments. Static microscale platforms are further categorized into four types: (1) Transwell platform, (2) microtunnel platform, (3) micropattern platform, and (4) wells-within-a well platform [6].

Transwell platforms are multicompartmental static systems, which were first conceptualized by Dr. Stephen Boyden in 1961 to analyze leukocyte migration [15]. A Transwell platform comprises a cylindrical insert having a thin porous polymeric membrane bottom. The Transwell can be placed inside a conventional cell culture well, which divides the well into two compartments. In the upper and lower compartments, different cells/organ models could be grown allowing an efficient exchange of medium, cell-to-cell contact, and cell migration through the microporous bottom of the Transwell insert creating interorgan interactions. This Transwell platform could be used to study cell invasion, migration, particularly helpful for studies that involve barrier tissues such as skin, intestinal wall, blood–brain barrier, and responses of the cells toward different drug compounds and their metabolites in the donor and receiver compartments [15]. For instance, a coculture system comprising Caco-2 and HepG2 cells was used to study the toxicity levels of the compound benzo[*a*]pyrene. Caco-2 and HepG2 cells in the coculture system mimic the human metabolic process that takes place through the intestinal absorption and cytochrome P450 mediated biotransformation occurring in both intestines and liver, respectively [16].

Microtunnel platform, on the other hand, creates horizontal microfabricated fluidic tunnels to make connections among the different organ chambers [6]. As a result of longer horizontal fluid tunnels and a smaller cross-sectional area of the fluid path, it leads to inefficient medium exchange but still can guide in cellular migration and growth of cellular projections. The neuronal interactions with cancer cells can be investigated using such microtunnel platforms where the neurons provide biophysical support and assist in the migration of cancer cells. This can be useful for in vitro screening of inhibitors that block the perineural invasion of cancer cells along the neurites [17]. Micropatterning techniques allow the creation of 3D pattern configuration of several smaller regional compartments or islands inside a single big compartment where multiorgan coculture can be carried out and is called the micropattern platform [18]. The smaller compartments comprise individual organ cells, spatially separated, and cell attachment is aided due to the surface modifications. Cellular interactions are promoted by the diffusion of the overlying medium and occasionally through direct physical contact with the neighboring cells [18]. A human hepatocyte model comprising primary human hepatocyte cells organized into 2D islands and surrounded by fibroblast cells was developed to unravel the host-pathogen interactions and hepatic life cycles of hepatitis B and C viruses and *Plasmodium* pathogens [19]. Another example of a micropattern platform is the coculturing of A549 lung cancer cells and osteoblast cells hFOB 1.19 using the m-eraser technology to create micropatterns on different substrates such as poly(lactic-co-glycolic acid) (PLGA) nanofiber sheets and tissue culture plate (TCP) simulating lung cancer bone metastasis. This micropattern model was used to

analyze the anticancer efficacy of chemotherapeutic drug doxorubicin and evaluate cellular communication to have an extensive understanding of metastatic states in lung cancer [20].

Finally, the last category of static microscale platforms is the wells-within-a-well platform. As the name suggests, a cell culture plate contains larger wells and within each of the wells contains multiple smaller wells that can be used to culture different organ cells simultaneously (one organ per well in their respective medium). A common culture medium is filled above the inner wells that will promote the diffusion of soluble metabolites and initiate cross-talk across the multiple organ compartments. The wells are separated by fabricating physical barriers instead of 3D micropatterns and overcome the limitation of interorgan interactions associated with conventional 2D cell culture systems. Li et al. developed the first commercial integrated discrete multiple organ cell culture (IdMOC) system to analyze the cytotoxicity of tamoxifen quantitatively on different primary human cells (liver, kidney, lungs, astrocytes for the central nervous system, endothelial cells for blood vessels, and a breast cancer cell line MCF-7) [21].

12.3.2 Single-Pass Microfluidic Platforms

Different organ models when physically integrated allowing all the organ units to receive sequential medium perfusion through an open-loop system are called a single-pass microfluidic platform. The unidirectional route of metabolites usually flows from the upstream organ units to the downstream organ units and thus maintains organ-organ interactions. In some models of the single-pass microfluidic platform, the presence of functional barrier tissue would require multiple loop system arrangements [6]. These single-pass microfluidic platforms usually provide a reliable qualitative pharmacodynamic evaluation of drugs due to the steady maintenance of nutrient supply through all the organ units, drug dose, and waste removal. Midwoud et al. constructed a polydimethylsiloxane (PDMS) based novel in vitro microdevice to test the metabolism of 7-ethoxycoumarin (7-EC) [21]. The PDMS-based single-pass microfluidic platform has microchambers of 25 μL volume to integrate precision-cut liver slices and the microdevice is connected to a perfusion system for the constant nutrient, oxygen supply, and waste removal. The metabolism of the test compound was found to be constant for about 3 h and was able to mimic the in vivo metabolic condition in an improved manner, paving the way for its potential application in drug development [22].

12.3.3 Pump-Driven Recirculating Microfluidic Platforms

An upgrade to the single-pass microfluidic platform is the pump-driven recirculating microfluidic platform, where a closed-loop system ensures circulating medium flow throughout the individually connected organ compartments. Cellular metabolites discharged from one organ can act on other organs through this closed-loop

circulation until cleared. Moreover, organ-organ interactions in a recirculating microfluidic platform can be achieved through serial, parallel, and combined perfusion systems. This system closely mimics the blood flow in the human body, which gives an idea about the pharmacokinetics of drugs. A three-compartment micro CCA (μCCA) based on a silicon chip was developed by Shuler and colleagues using microfabrication technology and coupled to a peristaltic pump for medium recirculation [23]. Later they extended this model to construct a four-chambered model integrating more parenchymal organs and also introduced 3D hydrogels into the (μCCA) platform to physiologically replicate the three-dimensional parenchymal tissue structures [24, 25].

Pump-driven recirculating microfluidic platforms are also created for functional barrier tissues like skin, intestines, and lungs, which are fabricated on microporous membranes separating the apical and basal space of the barrier tissues. A fine example of such microfluidic platforms that can incorporate both barrier and non-barrier tissues was conceptualized by Mahler et al. [26]. The team integrated a five-compartment Transwell-based multiorgan microphysiological (MOM) system that represents the gastrointestinal tract and four-compartment silicon (μCCA) while connecting them by tubing [26]. The gastrointestinal tract in the human body is mainly known for its absorption capacities and the essential nutrients, and chemicals that enter systemic circulation through the intestinal route. This integrated microfluidic platform aims to study the absorption, metabolism, and toxicity (ADME) of oral supplements as the platform mimics the systemic intestinal apical dual circulation and possibly could be an important tool to investigate the ADME profiles of orally consumed substances [26].

12.3.4 Pumpless Recirculating Microfluidic Platforms

The proof-of-concept pumpless recirculating microfluidic platform was proposed by Sung et al. to incorporate gravity-driven medium flow throughout the platform, thus eliminating the need for an external pump [27]. The group designed the novel platform in such a way that it would require minimal specialized fabrication techniques for the assembly and operation and would be helpful for nonexperts and industrial scalability to conduct high-throughput screening of drugs. This microfluidic platform embodied two aspects of MOM, one integrating 3D-based hydrogel cell culture in a μCCA to grow the three different cell lines representing tumor, liver, and bone marrow and the other developing a mathematical PK-PD approach quantitative estimation of the target compound. All the components of the platform including the organ microchambers, reservoirs, and microchannels were constructed on a chip without an external pump. The absence of an external pump prevents bubble formation. The medium recirculation was guaranteed on account of gravity-driven flow as the entire microfluidic device was positioned on a rocking platform that was tilted periodically. The continuous bidirectional medium recirculation did not affect the metabolic profile of the drug of interest that was used for toxicity assessment [27]. Based on this concept, several other models of pumpless recirculating microfluidic platforms have been developed so far, for example, the

construction of a fourorgan MOM comprising cardiac, liver, neuronal, and muscle modules [28], incorporation of barrier tissues like guton-a-chip [29], and a whole body-on-a-chip model. The whole body-on-a-chip model is based on PK-PD concept that includes multiple organs, barrier and non-barrier tissues separated from each other in the microfluidic platform [30].

12.4 Techniques of Fabrication

The integration of multiple organs into a body-on-a-chip platform ideates the possibility of achieving whole-body responses in the drug discovery process. Even though the technical expertise required to design and fabricate organ-on-chip (OOC)/body-on-chip (BOC) models is limited [31], the basic steps to build such platforms require four components: (1) a microfluidic chip, (2) 2D/3D microtissues cultured in a chip, (3) external factors such as culture medium, biophysical, mechanical, and electrical to nurture the microtissues, and (4) sensors to monitor physiological behavior and give readouts [32]. Once the design of the OOC/BOC is finalized, it is imperative to consider certain aspects in the fabrication process, such as maintaining the physiological relevance of the target organ, reproducibility, and a suitable fabrication technique specific for the experiment and organ of interest. Some other noteworthy key points to consider are as follows: (1) technical expertise, (2) time, resources, and expenses, (3) high-throughput screening, and (4) physical and material limitations that might affect the overall physiology of the cells/tissues being studied [33]. In this section, we will discuss some of the techniques currently used in the fabrication of OOC/BOC microfluidic platforms.

12.4.1 3D Bioprinting

The creation of 3D tissue constructs by the layering of living cells and scaffolds using a printer and bio-inks to emulate the cellular architecture of human tissues/organs is called 3D bioprinting [34–36]. The basic method of 3D printing is the additive technique of layer-by-layer assembly to create the final complex three-dimensional structure and this is useful in the creation of microphysiological systems such as OOC and BOC. Several components of an OOC/BOC could be printed using 3D printing and the individual tissue scaffolds could be embedded into the microfluidic device. The entire process of printing is automated, which reduces time in fabrication and maintains replicability owing to the use of computer-aided design (CAD) programs [37]. 3D bioprinting is divided into three categories: (1) microextrusion bioprinting, (2) inkjet bioprinting, and (3) laser-assisted bioprinting to produce both natural and synthetic scaffolds that incorporate various other additives such as growth supplements/chemicals and the biomaterial (bio-ink). The advantages associated with the applications of bioprinting in the fabrication of OOC and BOC are intriguing but require optimization to mitigate some of the constraints; for instance, degrees of heat and pressure in the bioprinting process often damages the polymer and bio-ink affecting the final product [1].

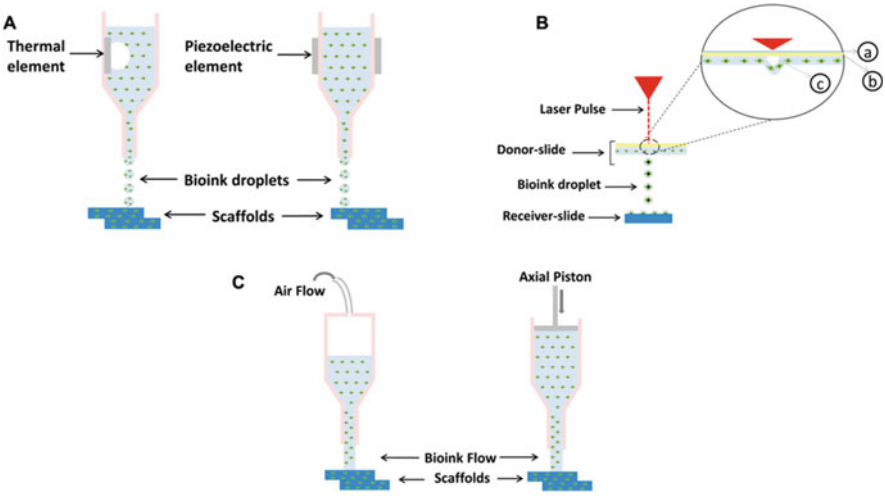

Fig. 12.2 (**A**) Schematic of inkjet-based bioprinting. Thermal inkjet uses heat-induced bubble nucleation that propels the bio-ink through the micro-nozzle. Piezoelectric actuator produces acoustic waves that propel the bio-ink through the micro-nozzle. (**B**) Schematic of laser-assisted bioprinting: (a) transparent glass, (b) thin metal layer, and (c) vaporization-induced bubble. Bubble nucleation induced by laser energy propels droplets of bio-ink toward the substrate. This technique has a minimal effect on cell viability. A receiver-slide can be a biopaper, polymer sheet, or scaffold. (**C**) Schematic of extrusion-based bioprinting; left, pneumatic based; right, mechanical based. Struts are extruded via pneumatic or mechanical pressure through micro-nozzles. Extrusion-based techniques can produce structures with great mechanical properties and print fidelity. (Adapted and Copyright from Kačarević, Ž.P.; Rider, P.M.; Alkildani, S.; Retnasingh, S.; Smeets, R.; Jung, O.; Ivanišević, Z.; Barbeck, M. An Introduction to 3D Bioprinting: Possibilities, Challenges and Future Aspects. Materials 2018, 11, 2199. https://doi.org/10.3390/ma11112199 [51]. Under Creative Commons Attribution License)

Inkjet bioprinting was developed in 2003 where microdroplets of bio-ink are projected onto a substrate to form 3D structures [38]. It can be used to fabricate two important components of an OOC/BOC, that is, the microfluidic chip and the microtissues [32]. Inkjet bioprinting is a contactless procedure to create a layer-by-layer assembly of the bio-ink that is stored in a cartridge [39] (Fig. 12.2A). Temperature manipulation in the heater or piezoelectric forces create microbubbles that result in the propulsion of the bio-ink as a droplet on a receiving substrate [39], thus creating a variety of complex microtissues such as skin, blood vessels, liver, and lung [40, 41]. This entire procedure involves low costs, high reproducibility and quality, and minimal time and offers the flexibility to work with several materials.

Unlike inkjet bioprinting, laser-assisted bioprinting (LAB), laser energy mostly relies on nanosecond lasers or near UV (ultraviolet) wavelength laser source to propel the bio-ink like a gel ribbon onto a receiving layer making high-precision tissue structures [42, 43]. The pulsed laser source, biological materials that are shaped into ribbons, and the receiving platform comprising cell growth medium/biopolymer are three components of this technique [44] where the laser energy

evaporates the flowing liquid bio-ink causing it to form droplets and the cells duly adhere to the substrate allowing them to grow into the desired target microtissue (Fig. 12.2B). Factors that affect the resolution of the printed material include laser energy (high energy might damage the biological material), substrate wettability, speed of printing, rheology, and thickness of biological materials [45, 46]. Examples of cells that have been successfully printed using LAB are human dermal fibroblasts, mouse myoblasts, neural stem cells, tumor cells like MCF-7, to name a few [47, 48].

Microextrusion-based bioprinting in contrary to both inkjet and LAB is a common bioprinting technique to conduct the fabrication of tissue structures that expels the bio-ink from a micronozzle as a continuous stream and deposits it on a receiving substrate [34, 49] (Fig. 12.2C). One of the primary advantages associated with microextrusion bioprinting is the ability to work with highly viscous bio-inks and printing could be done continuously, which results in the making of high integrity microtissues. However, due to the high pressure required to propel the continuous high viscosity biological material, it may lead to compromise in the cell viability, which is a factor needed to be considered while designing the experiments [50].

12.4.2 Stereolithography

Stereolithography is a solid free-form additive manufacturing technique introduced in 1986 by Charles W. Hull [52] and has gained immense popularity in the domain of microfluidic fabrication technologies over the recent years. This technique uses UV laser or light to illuminate the liquid polymer material, which cross-polymerizes eventually creating layer-by-layer assembly to form solid structures (Fig. 12.3). Stereolithography produces high integrity and accurate microfluidic chips for organ-on-chips (OOCs), affordable and compact, and has been in continuous innovation to offer these advantages [37]. Owing to its nozzle-free printing approach, stereolithography is a faster technique than its nozzle-based counterparts due to its assigned movement only in the *Z*-direction. Additional benefits of using stereolithography for the construction of OOC/BOCs include reduced shear stress on the living cells, no requirement of high viscosity bio-inks, and use with a diverse range of materials [53, 54]. Stereolithography has been adapted to develop a layerless procedure to create continuous polymeric parts using an oxygen-permeable source that inhibits photopolymerization at the surface called the "dead zone" while drawing out complex solid parts at a faster rate [55]. Since this technique is a sought-after procedure specifically for the construction of OOCs, extensive research has been conducted also in the customization of the materials such as resins and combined with other techniques like soft lithography [56] to fabricate 3D functionalized perfusion network [57], multicellular spheroid culture device [58], implantable porous membrane barriers [59], and lung-on-chip platform to investigate the role of inflammation-induced thrombosis [56].

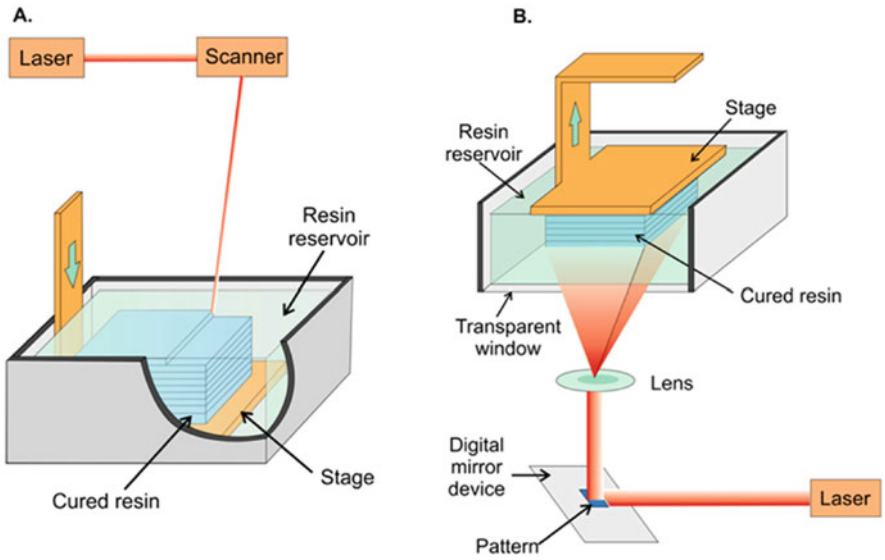

Fig. 12.3 (**a**) Schematic of a bath configuration SLA printer with a direct write curing process. The stage is located just below the surface of the liquid resin. A single laser moves along the surface of the resin, row by row, until completely curing the desired layer. To initiate the following layer, the stage sinks lower into the vat until a new layer of liquid resin covers the surface and the curing process repeats. (**b**) Schematic of a layer configuration SLA printer with a projection-based curing method. In this particular configuration of an SLA printer, the stage is submerged a defined distance into the photopolymer reservoir. Next, a laser is guided to the stage to polymerize the material in the reservoir that is between the laser and the stage. In the projection-based curing method, the digital mirror device allows for a whole layer to be cured simultaneously. The stage can then be raised again by a defined distance, and another layer can be cured. This procedure repeats until the object is printed. (Reprinted (adapted) with permission from (Bethany C. Gross, Jayda L. Erkal, Sarah Y. Lockwood, Chengpeng Chen, and Dana M. Spence *Analytical Chemistry* **2014** *86* (7), 3240–3253. https://doi.org/10.1021/ac403397r [60]. Copyright (2014) American Chemical Society)

12.4.3 Injection Molding

Injection molding is prevalently used for the large-scale production of one of the crucial elements in OOCs is the fabrication of the microfluidic device [37]. The entire process is divided into four main steps: (1) melting of materials, (2) compression of molds together, (3) injection of the target material into the respective molds, and (4) cooling and removal of the mold cavity [61]. A primary example of the injection molding technique was applied to the fabrication of a liver-on-chip model to study the hypothermic storage ability. This study aimed to understand and develop a strategy to preserve liver-on-chip models on account of their cell viability, tissue morphology maintenance, and biotransformation capacity and how can it be easily accessible to researchers in the biomedical research community [62]. Though it is a fairly easy-to-use technique requiring skills and experience [37], it has certain

disadvantages such as its limited capacity to use a variety of materials and simplified mold designs [63]. The technique can advance from its current state by reducing time and costs and also paves the way to include diverse materials [61]. Szydzik et al. [64] developed an on-chip valve-based microfluidic module for automated fluidic handling for point-of-care diagnostic biosensor devices by integrating a PDMS-based injection molding fabrication technique. This integrative innovation thus allows for the practical solution to bring about mass production of automated point-of-care diagnostics and highly-sensitive biosensor assays in the realm of biomedical research.

12.4.4 Soft Lithography

Soft lithography is an extension of the method of photolithography where it employs a myriad of elastomeric materials to fabricate 3D structures and is a popular, reliable technique for the construction of OOCs [65]. The name "soft" associated with this technique is attributed due to the ability to use mechanically softer materials such as polymers and gels in the fabrication process. One of the widely used materials in soft lithography is polydimethylsiloxane (PDMS) because of its high biocompatibility, transparency, easy handling, versatile surface chemistry, durability, and low costs [66]. All these features make PDMS the go-to material for the creation of microfluidic devices. Soft lithography encompasses several fabrication techniques such as replica molding, capillary molding, microcontact molding, and micro-transfer molding that are all based on the usage of individual patterned PDMS layers similar to the exposed layer [65].

In replica molding, soft biocompatible polymers like agarose can be poured into the micropatterned PDMS layer, then left for polymer curing, and finally employed to remove the imprinted polymer from the PDMS mold (Fig. 12.4). In the case of capillary molding, patterned PDMS mold is brought into the contact of any substrate and liquid polymer is poured to fill the patterns of the mold on account of capillarity. The PDMS mold is removed after curing, leaving desired microstructures on the substrate. In microcontact molding, PDMS acts as a stamp, which is soaked in the ink such as living cells, small molecules, and protein molecules and later brought into contact with the substrate, thus transferring only the ink to the substrate. In micro-transfer molding like replica molding, the patterned PDMS mold can be filled with a liquid polymer, followed by the removal of excess polymer and finally transferring to a substrate of choice. The PDMS layer is then gently peeled away from the substrate leaving behind an imprint of the solid structure [65].

Even though PDMS has a lot of versatile salient features in the soft lithography process, it is limited by the bio-resistive nature that leads to the adherence and absorption of living cells/tissues, proteins, and drugs onto its walls, thus interfering with the accuracy of results [1]. Possible solutions to this problem are the development and widespread use of high fluorinated elastomers that alleviate inertness and improve biological resistivity and the improvement in the mechanical properties by mixing the PDMS polymer with curing agents at a desirable ratio. The second

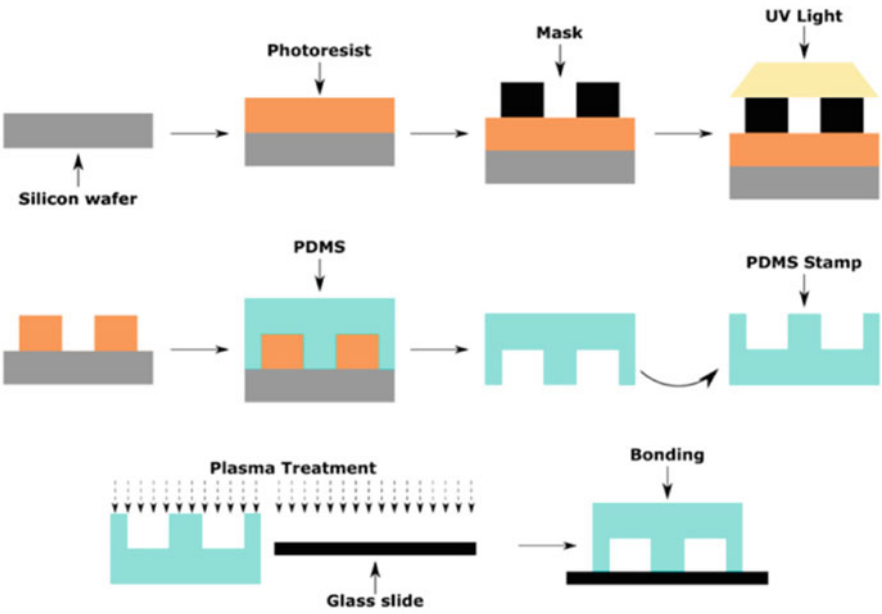

Fig. 12.4 An example of replica molding using polydimethylsiloxane (PDMS). The typical photolithography steps are taken utilizing photoresist, a mask, and UV exposure to create the master. The master mold is filled with PDMS and cured. Once cured, the PDMS is removed from the master and bonded to a glass slide for plasma oxidation. (Adapted and Copyright from Puryear Iii, J. R., Yoon, J. K., & Kim, Y. (2020). Advanced Fabrication Techniques of Microengineered Physiological Systems. Micromachines, 11 (8), 730. https://doi.org/10.3390/mi11080730 [1]. Under Creative Commons Attribution License 4.0)

method has found success in the fabrication of numerous OOCs [67], for example, heart-on-chip, lung-on-chip [68], and liver chip platforms [69].

12.5 Pros of Body-on-Chip

Drug development is an arduous journey starting from the concept of drug designing to selecting the candidate drug molecules for their in vitro and in vivo evaluations, eventually leading up to the approval by the Food and Drug Administration (FDA) for human use. As discussed in the earlier sections regarding the importance of drug testing in in vitro and in vivo conditions, the outcome from these tests decides their fate in the further step whether they will be carried forward to the clinical trial phase or not. A large amount of these drug candidates does not always end up showing the same favorable results in the clinical trials as opposed to what they showed in the in vitro and in vivo tests, leading to attrition. Organ-on-chip platforms were the initial go-to option for researchers to investigate diligently the ADME of drugs in a controlled environment. However, they comprise only a specific organ in the

microfluidic platform, which is not enough to give a bigger picture of the entire drug metabolic activity as the human physiological environment relies on the dynamism of intra/interorgan interactions. Body-on-chip microfluidic devices in this advanced era of biomedical technologies promised a ray of hope to understand and mimic the human microenvironment much closer than to what organ-on-chip and animal models delivered so far. If they are designed carefully keeping in mind all the salient features of the human body, these MOM platforms would predict the drug responses and its metabolism in a much similar manner and can provide alternatives to refrain from using animal models soon.

Body-on-chip systems are constructed in such a way to faithfully mimic the in vivo organ microenvironment to represent the intra/interorgan dynamic cross-communication, different tissues could be fabricated together to understand their individualistic responses to the same drug, and can be tailored to disease-specific conditions of different patients, thus also catering to the need for personalized medicine. These systems will help to speed up the research time due to the ability to screen different concentrations of the same drug at the same time to investigate its therapeutic concentration. Since these systems are manufactured at low costs, there won't be the financial burden of using them repeatedly for the drug testing process in the initial rounds of the drug discovery process, which in general is incurred more in the case of animal models. This will reduce the usage of animal models and also respect the ethical rights of sentient beings. Body-on-chip devices are manufactured at a large scale and are easy to use, compact, and portable and henceforth these microfluidic systems are the modern-day invention that brings several advantages and gives much-needed impetus to the current drug development process for reducing failures in a clinical trial and drug attrition rates.

12.6 Applications of Organ-on-a-Chip Technologies

12.6.1 Drug Discovery

In vitro 2D cell culture systems have seen progress over the years since their invention through the substantial knowledge acquired from the disparate branches of biological sciences [42]. The technique for two-dimensional (2D) cell culture protocol is seeding the cells in nutrient and antibiotic rich media and maintaining them at a physiological temperature of 37 °C. The confluent cells were employed in important drug studies, and the results obtained determine the fate of the drugs in advancing to clinical trials [70]. However, these 2D models are incapable of recapitulating the complex microenvironment of the cellular microenvironment. The animal-based models are more thorough in emulating the human biophysiological functions. The interspecies differences and the expense involved stand as a barrier in further drug screening and therapy [71]. The realization that OOC/BOC can possibly reduce the cost and to some extent the negative results of the existing 2D and animal-based development programs can accelerate the identification and optimization of new drugs in the pharmaceutical fields. The microfluidic

system can revolutionize the platforms to control cellular environment with high precision, and the signals such as mechanical, chemical, and electrical can be applied to the cellular paradigms [72]. In 3D culture, different environmental cues provide a match to the human pharmacokinetic and pharmacodynamic profiles to study drug ADME processes. The cross-talk between the cells in 3D culture systems accurately represents differentiation and proliferative functions to mimic various pathophysiological conditions such as tumor microenvironment. In a recent study, 3D cell culture systems demonstrated resistivity to cancer drug cisplatin and generated a high concentration of reactive oxygen species (ROS) contrary to the 2D culture [73]. One of the most important components of drug discoveries includes nephrotoxicity. The kidney-on-a-chip model has been modified precisely to investigate the cellular and protein interactions that take place inside the glomerulus, thus assisting to comprehend the nephrotoxicity process of certain drugs [74]. However, the construction of microfluidic/3D technologies using glomerulus disregards many of its structural components and is still under progress. The integration of fluid endothelium lining and salt balancing nephron into a single glomerulus apparatus was not possible, restricting the consistency of data with previous studies. This probably occurred because the multiorgan constructs contain a limited cell lineage per organ [42]. Discoveries have also been made in the advancement of drug screening using lung-on-a-chip models as 2D and animal models are powerless in studying vasculopathy, altered homeostasis, and human lung physiology.

In vitro models cannot exhibit thrombosis in lung microvessels nor evaluate toxicological–therapeutic antithrombotic drugs. Addressing this, a microfluidic device was constructed demonstrating thrombosis induced by lipopolysaccharide (LPS) mediated-inflammation in the human type I and II primary alveolar epithelium [75]. The device is equipped with a perfusion system of whole blood input. This microfluidic chip recapitulates in vivo responses by indirectly activating intravascular thrombosis through lipopolysaccharide (LPS) endotoxins via epithelium interaction instead of the suspected alveolar endothelium. This lung-on-chip model was able to demonstrate antithrombotic therapeutics by inhibiting the mediator of tissue inflammatory protein protease-activated receptor-1 (PAR-1), which is also involved in maintaining hemostasis and activating aggregation of platelets. This sort of microfluidic platform is a useful tool to dissect the cytoprotective and antithrombotic effects of parmodulin-1 (PM2), an antagonist of PAR-1 in preventing acute lung injury and blood perfusion. Thus, new antithrombotic drugs can be preclinically tested for off-target thrombotic effects using the lung-on-a-chip tool [75]. Orally administered drugs are primarily screened at the intestine assessing their ADME profile. PDMS and hydrogel-based microfluidic devices seeded with epithelial cells are developed to mimic the drug transport across barrier tissues such as intestinal wall and epithelium. These devices are also designed to incorporate functional structures like microscopic villi [76]. Curcumin was used to study the intestinal permeability in Caco-2 cells cultured on a membrane-based microfluidic device in a real-time manner. The data generated from the mass spectroscopic analysis correlated with the previous findings on the human intestinal barrier function [77]. Coculturing of intestinal bacteria can be utilized in the investigation of the

functional role of the gut microbiome in the drug absorption process. This enables a gnotobiotic environment to reveal the cross-talk between multicellular interactions that involve microbiome and immune elements, hence providing an opportunity for developing therapeutics immunocompromised disorders like inflammatory bowel disease (IBD) [78].

OOC models are recruited for prediction and mechanistic investigation of prospective drugs accurately and mitigation of their toxic effects through advanced therapies. The predictions produced by these models could inform and facilitate early efforts in the diagnosis, prognosis, and treatment of various diseases by developing safer drugs with an improved prospect of triumph in clinical trials and cost-effectiveness [79].

12.6.2 Cardiovascular Diseases

Cardiovascular diseases (CVD) are multifactorial diseases including coronary artery diseases (CAD), hypertension, stroke, congenital heart diseases, and vascular diseases leading to increased mortality worldwide. Change in lifestyle, environmental factors, and genetic predispositions are a few of the contributing risk factors [80]. The contractile and electrophysiological activities of the heart tissue are maintained by the contracting and polarized cardiomyocytes that rely on the external physiochemical stimuli [72]. The ideology of heart-on-a-chip was developed owing to the dissimilarity between animal and human hearts aiming to study the various heart diseases and cardiotoxicity of chemical treatments [81]. This technology can help decipher the functions of the human heart, thereby developing appropriate disease and drug screening heart models, simulating tissue structures and functions at the micron level, and bridging the gap between in vitro and in vivo systems [82].

The variation in calcium ion concentration plays an important role in both short- and long-term critical repercussions of ischemia ranging from arrhythmia to heart failure. A study conducted by Martewicz et al. [83] using confocal microscopy and heart-on-a-chip confirmed that calcium dynamics were altered when neonatal rat cardiomyocytes loaded in Fluo-4 reached a state of hypoxia. The calcium ions were also recovered to a normal level after reversing to normoxic conditions, establishing the reversibility of the biological effects. This reversibility of calcium transits represents an adaptive mechanism for cardio preservation in ischemia improving the survival of patients. Agarwal et al. [84] constructed a heart-on-a-chip microfluidic system using a semiautomatic fabrication technique processed sub-millimetre soft-elastomeric thin-film cantilevers and engineered anisotropic cardiac cells onto the cantilevers. Diastolic and systolic stresses were detected based on the deflection of these cantilevers during muscle contraction. Cardiac contractility was measured using this tool after treatment with isoproterenol at a dosage from 1 nM to 100 μM and the positive inotropic effect was analyzed. This study was able to exhibit the competence of these devices in assessing various concentrations of drugs. Another study verified the effect of shear stress within the PDMS-based microfluidic chip system in regulating the transformation and

formation of aortic valvular interstitial cells and focal adhesion, respectively. The cells were found to elongate and align with the flow at the physiologically relevant shear stress level [85].

Marsano et al. [86] designed a heart-on-a-chip platform using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) to generate micro-engineered cardiac tissues (μECTs). They were able to observe that the stimulated μECTs displayed superior cardiac differentiation compared to the control. This model also established spontaneous and synchronous beating of the cardiomyocytes as well as the expected response to isoprenaline, a β-adrenergic-agonist. The I-Wire heart-on-a-chip model was created using a PDMS casting mold. The PDMS mold was placed inside a 6-well plate having channels to accommodate titanium-based anchoring wires [87]. Engineered 3D cardiac tissue constructs (ECTCs) potentially replicate complex cardiac physiology, and hence neonatal rat cardiomyocytes in the presence of fibrin were cultured in the PDMS molds. The I-Wire heart-on-a-chip model not only controls the force applied to the ECTCs but also enables to study the electromechanical characterization of the ECTCs using an inverted microscope and measure the synchronized contractions. This model would prove to be beneficial to comprehend better cardiac diseases and the related drug screening process to develop advanced regenerative therapeutics [87]. Cardiac ischemia accounts for a large part of the population suffering from CVD. Arterial blockade drastically reduces blood flow to the heart muscle causing a hypoxic region in the downstream muscle. An integrated extra- or intracellular bioelectronic device to represent heart ischemia was fabricated to comprise a cell culture area for culturing HL-1 cells and microfluidic channels [88]. The extracellular device recorded the highly stable signals and monitored the frequency of the reduction in beats and wave propagation that often occurs in hypoxic cells. The intracellular devices record the action potential (AP)-like readouts. Oxygen deficit stimulates ATP-dependent K^+ channels promoting repolarization of the membrane, which seems to be correlating with narrowed AP readouts during hypoxic conditions. This ischemic model in addition to detecting hypoxia also incorporated other factors involved in ischemic conditions such as nutrient deprivation, acidosis, and accumulation of wastes by modulating medium composition and flow [88]. The heart-on-a-chip model may not represent the actual human heart but is one step ahead in the pharmaceutical field of developing cardiac disease models and drug screening platforms. The technique involves simple and accurate measuring methods and is cost-effective compared to other classical devices [82].

12.6.3 Antiaging Medicine

One of the common aspects of senescence involves natural skin aging with obvious shreds of evidence in course of time. This process encompasses various complex biological procedures involving the endogenous and exogenous factors that contribute to the structural and physiological modifications in each layer of the skin as well as its appearance [89]. Endogenous aging occurs as part and parcel of chronological

aging that is influenced by genetics, hormonal fluctuations, and various metabolic process. Deficiency in DNA repair mechanisms and DNA damage responses contribute to premature aging. Intrinsic factors for DNA damage include spontaneous hydrolysis, replication mistakes, free radicals, and RO generation [90]. On the other hand, premature aging is also caused by the direct contact of the skin with multiple external factors. Ultraviolet (UV) radiation is considered as one of the fundamental exogenous factors that cause premature aging leading to photoaged skin with thickened epidermis, mottled discoloration, deep wrinkles, dullness, and roughness [91]. A microscale microfluidic system was characterized by Wen et al. [92] to study the effect of polydatin natural resveratrol in antiaging properties on *Caenorhabditis elegans* (*C. elegans*) experiencing oxidative stress and heavy metal exposure. A series of functionalized units facilitated individual worm's loading, long-term culture, immobilization, and imaging in the device. Owing to the unique design of the microdevice, long-term maintenance of the worms is possible for up to 35 days with sufficient nutrient exchange. The drug polydatin significantly extended the worm's life span up to 30.7% and 62.1% under normal and stress conditions, respectively. The Daf-16 forkhead transcription factor is important in the insulin/IGF-1 signaling pathway regulating development, longevity, metabolic functions, and stress resistance in *C. elegans* also similar to human beings. Superoxide dismutase (SOD)-3 is a downstream effector of Daf-16, which has the power to reverse the aging process by decreasing the free radical production and preventing oxidizing of sensitive biological molecules. Quantitative real-time polymerase chain reaction (PCR) analysis revealed that polydatin drug led to the downregulation of Daf-2 signaling leading to the translocation of Daf-16 forkhead transcription factor into the nucleus and also increase in the SOD-3 expression. The use of the microfluidic system made the possibility to monitor the lifelong and real-time tracking of the multiple responses of individual *C. elegans* being exposed to external stimuli. This provided a useful tool for whole animal assay and antiaging drug evaluation at a single animal resolution [92].

In senescent cells, pathways that are up- or downregulated involve p16, p21, and p53 signaling. The identification and isolation of senescent cells for radiation biodosimetry can be conducted based on their physical properties using microfluidic techniques. Chen et al. [93] developed a senescent chip using a slanted micropillar array with an inclined angle relative to the fluidic flow (between 0° and 90°). This 3D microfluidic configuration was shown to isolate and analyze senescent mesenchymal stem cells (MSCs) from undiluted human whole blood and irradiated mouse bone marrow, respectively. This technique focuses on dead-end flow and cross-flow filtrations to separate senescent cells by preventing cell clogging and cellular damage. This study was found to be potential for biological and clinical applications for antiaging therapy "senescent dialysis" as an innovative technique to extricate senescent cells from human whole blood without affecting the white blood cells during filtrations.

12.6.4 Cancer

Typically, cancer research involves the extrapolation of in vitro 2D cell culture studies and animal models investigating the tumor pathophysiology [94]. In the past decades, the screening of anticancer drugs, and understanding the cell signaling pathways, proliferation, migration, reaction to the drug including protein/gene expression were conducted using in vitro models [95]. However, the in vitro models cannot provide appropriate feedback in mimicking the tumor environment, thus failing to guide with proper insight on the effect of complex spatial cellular organization and interaction [96]. On the contrary, animal models offer in-depth knowledge about tumor growth and drug responses in vivo, but are highly expensive, and interspecies variation occurs [97]. Microfluidics and organ-on-a-chip/body-on-chip technologies can pave the way for modern and effective approaches in recapitulating the tumor cell organization, screening of the anticancer drugs, and thereby improving cancer therapy [98].

Brain metastases are the most lethal cancer lesions, and an 20% of all cancer patients are estimated to develop them. Despite the multimodal treatment and advances in systemic therapies, still the prognosis remains poor. In the brain tumor environment, a healthy and intact blood–brain barrier (BBB) is limiting drug access to the tumor cells [99]. Advanced therapies are required to understand the mechanisms that drive brain metastases and improve the bioavailability of the drug beyond the BBB so that they can be targeted, minimize invasive surgical therapies, and have better outcomes for the patients. The development of new tools that can measure the extravasation and migration through an endothelial barrier into brain tissue poses a leap into identifying brain-metastatic characteristics [100]. Silvani et al. [101] developed glioblastoma multiforme (GBM)-on-a-chip model comprising sealed microfluidic channels, lined with endothelial cells mimicking the BBB, integrated with a 3D bioprinter GBM model. The 3D bioprinting technology allowed the embedding of the selected cells in specific engineered hydrogels with stiffness and the microfluidic channels promoted the controlled perfusion at the physiological flow rates required for the maturation of BBB and drug injection with images captured using confocal microscopy. The combination of simulated microgravity (μG) with the GBM-on-a-chip model inhibited the spontaneous aggregation of the GBM cells into the surrounding microenvironment within 3 days of exposure. The active YAP protein expression essential for the cancer cell survival and functionality deteriorated demonstrating that gravitational drive mechanotransduction and lack of gravity compromises and disrupts GBM cellular function and mechanism. Additionally, the actin cytoskeleton changed, and vinculin protein expression was reduced within 48h of exposure to μG. The drop in the tight junction protein zo-1 increased the cellular permeability with a dysregulated barrier function. This GBM model addressed the challenge of successful chemotherapeutics delivery across the BBB.

Breast cancer is the most frequent and second leading cause of malignant cancer in women. Breast cancer is metastatic cancer and its development involves multiple steps process of involving multiple cell types [102]. Ductal carcinoma in situ (DCIS) is the accumulation of highly proliferative neoplastic epithelial cells in the lumen of

the mammary duct during the early stages of breast cancer [103]. Choi et al. detected early-stage breast cancer by fabricating a 3D microfluidic device mimicking the structural organization of the human mammary duct [103]. The device included a compartmentalized 3D microfluidic device containing cocultured human mammary duct epithelial cells and mammary fibroblast cells to generate breast tumor spheroids for drug screening purposes. For the growth and maintenance of the mammary epithelial cells, continuous flow of culture media (2–70 μL/h) was provided at the upper microchannel and DCIS spheroids were cultured on the upper layer of the extracellular matrix (ECM) membrane. On the lower layer of the ECM membrane, mammary fibroblast impregnates a stromal layer, which is perfused with culture media through the lower microchannel that mimics the vascular compartment of capillaries in the mammary stroma. A collagen membrane separated the upper and the lower microchannel. A continuous flow of the anticancer drug paclitaxel solution through the lower microchannel was generated. The effect of paclitaxel in this microfluidic device demonstrated the arresting of tumor cell proliferative capacity and inhibiting of DCIS growth in the tumor microenvironment.

These microfluidic platforms are accessible for the solid tumor microenvironment as well as to model liquid tumors. For example, Zheng et al. [104] designed an in vitro assay to study leukemia-induced bone marrow angiogenesis. An established microchip device replicating angiogenesis was altered to infuse leukemia cells on one side. On the other side, endothelial cells were seeded and allowed to emerge in the central chamber filled with collagen. The growth of endothelial cells was diverted toward the collagen matrix via the leukemic channel, suggesting angiogenesis of the leukemia cells. The control group showed the minimal invasive nature of endothelial cells into the collagen matrix due to the absence of leukemic cells. The linking of microfluidic systems with multiorgans is advantageous in understanding their compatibility with online analytical modules to monitor the organoid status in real time. One such study was conducted by Zhang et al. [105], demonstrating a cancer-on-a-chip platform combining liver cancer and cardiac organoids. The device comprises a fully integrated modular physical system to monitor temperature, oxygen, and pH along with biochemical sensors for biomarker detection, and an optical sensing platform for organoid morphology imaging through a fluidics-routing breadboard, operating the organ-on-a-chip units in a continual, dynamic, and automated manner. This system was created to simulate the effective dosing of doxorubicin (DOX) in vivo. The monitoring of anticancer effect and organ toxicity in a dual-organ human liver-cancer-and-heart-on-a-chip platform administered with DOX for up to 24 h was accurately mimicked by the microfluidic system. The cancer-on-a-chip models are obliged to be able to reproduce the complexity of each individual patient to stand for the genetic heterogeneity and simultaneously be easily accessible with lower complexity to maintain the clinical relevance. The integration of multiple organoids for tailored treatments is necessary for the account of the cross-talk between different tissues. The incorporation of parallelized characterization for the high-throughput screening of customized therapeutic drugs is still in its initial stage. This methodology offers an uncomplicated mode of assessing the most convenient treatments and drug concentrations [106].

12.7 Limitations of Organ-on-a-Chip/Body-on-a-Chip

Technical and entrepreneurial challenges are always intertwined with the development of the OOC/BOC models, etc. The synthetic materials used for the preparation of these models (PDMS, polycarbonate, polyester) often do not mimic the extracellular matrices appropriately in vivo conditions. This was recognized when PDMS showed absorption of hydrophobic molecules causing lowered pharmacological activity due to reducing effective drug concentrations [107]. Poor sensitivity in detection for analysis of biological responses also plays a key role as a technical issue owing to the low culture volumes and limited cell numbers in these models. This challenge can be modified in the bioanalytical platforms micro-engineered with miniaturized optics and sensors enabling high-resolution biochemical analysis even with reduced sample volume [108]. The inner ear is a complex organ that contains auditory sensory and supporting cells within perilymphatic and endolymphatic compartments. The complex intercellular interaction that occurs in the inner ear poses a challenging risk in the development of 3D models. Apparently, the lung 3D model currently used lacks various functions, including inflammatory-mediated response interacting with the lung epithelium, biomechanical ventilation, and the immune system active in the lung tissues [42]. Addressing these issues serves immense potential for 3D models in pharmaceutical markets for diversifying the prediction of clinical trial outcomes. To achieve an ultimate human-on-a-chip goal, laboratories are occupied in developing more mechanically active organ-on-chip models through microfluidics technology to deliver all-inclusive biomimetic techniques for physiology [109].

12.8 Conclusion

In conclusion, organ-on-a-chip has captured scientists globally improving the outlook of preclinical trials in drug discovery. The designing and preparation of organ-on-a-chip on an array of human organs has been conducted focusing on the ultimate goal of building a more complex multiorgan chip model, succeeding in the "Human-on-a-chip" model. In the long run, personalized drug screening will become a reality with strong standardization and reliability. The resolutions can generate interpatient variability in drug responses alleviating extensive and futile drug treatments and curtailing drug toxicity.

References

1. Puryear JR III, Yoon JK, Kim Y (2020) Advanced fabrication techniques of microengineered physiological systems. Micromachines 11(8):730. https://doi.org/10.3390/mi11080730
2. Cook D, Brown D, Alexander R et al (2014) Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. Nat Rev Drug Discov 13(6):419–431. https://doi.org/10.1038/nrd4309

3. Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J (2014) Clinical development success rates for investigational drugs. Nat Biotechnol 32(1):40–51. https://doi.org/10.1038/nbt.2786
4. Wong CH, Siah KW, Lo AW (2019) Estimation of clinical trial success rates and related parameters. Biostatistics 20(2):273–286. https://doi.org/10.1093/biostatistics/kxx069
5. Sung JH, Shuler ML (2009) A micro cell culture analog (μCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs. Lab Chip 9(10):1385. https://doi.org/10.1039/b901377f
6. Wang YI, Carmona C, Hickman JJ, Shuler ML (2018) Multiorgan microphysiological systems for drug development: strategies, advances, and challenges. Adv Healthc Mater 7(2):1701000. https://doi.org/10.1002/adhm.201701000
7. Peters SA (2012) Physiologically-based pharmacokinetic (PBPK) modeling and simulations. Wiley, Hoboken, NJ. https://doi.org/10.1002/9781118140291
8. Sweeney LM, Shuler ML, Quick DJ, Babish JG (1996) A preliminary physiologically based pharmacokinetic model for naphthalene and naphthalene oxide in mice and rats. Ann Biomed Eng 24(2):305–320. https://doi.org/10.1007/BF02667357
9. Shuler ML, Ghanem A, Quick D, Wong MC, Miller P (1996) A self-regulating cell culture analog device to mimic animal and human toxicological responses. Biotechnol Bioeng 52(1):45–60. https://doi.org/10.1002/(SICI)1097-0290(19961005)52:1<45::AID-BIT5>3.0.CO;2-Z
10. Quan Y, Sun M, Tan Z et al (2020) Organ-on-a-chip: the next generation platform for risk assessment of radiobiology. RSC Adv 10(65):39521–39530. https://doi.org/10.1039/D0RA05173J
11. Dehne EM, Marx U (2020) Human body-on-a-chip systems. In: Organ-on-a-chip. Elsevier, Amsterdam, pp 429–439. https://doi.org/10.1016/B978-0-12-817202-5.00013-9
12. Esch MB, King TL, Shuler ML (2011) The role of body-on-a-chip devices in drug and toxicity studies. Annu Rev Biomed Eng 13(1):55–72. https://doi.org/10.1146/annurev-bioeng-071910-124629
13. Sutherland ML, Fabre KM, Tagle DA (2013) The National Institutes of Health Microphysiological Systems Program focuses on a critical challenge in the drug discovery pipeline. Stem Cell Res Ther 4(S1):I1. https://doi.org/10.1186/scrt361
14. Perestrelo A, Águas A, Rainer A, Forte G (2015) Microfluidic organ/body-on-a-chip devices at the convergence of biology and microengineering. Sensors 15(12):31142–31170. https://doi.org/10.3390/s151229848
15. Boyden S (1962) The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J Exp Med 115(3):453–466. https://doi.org/10.1084/jem.115.3.453
16. Hyung Choi S, Nishikawa M, Sakoda A, Sakai Y (2004) Feasibility of a simple double-layered coculture system incorporating metabolic processes of the intestine and liver tissue: application to the analysis of benzo[a]pyrene toxicity. Toxicol In Vitro 18(3):393–402. https://doi.org/10.1016/j.tiv.2003.09.010
17. Lei Y, Li J, Wang N et al (2016) An on-chip model for investigating the interaction between neurons and cancer cells. Integr Biol 8(3):359–367. https://doi.org/10.1039/c5ib00309a
18. Nakanishi J, Takarada T, Yamaguchi K, Maeda M (2008) Recent advances in cell micropatterning techniques for bioanalytical and biomedical sciences. Anal Sci 24(1):67–72. https://doi.org/10.2116/analsci.24.67
19. March S, Ramanan V, Trehan K et al (2015) Micropatterned coculture of primary human hepatocytes and supportive cells for the study of hepatotropic pathogens. Nat Protoc 10(12):2027–2053. https://doi.org/10.1038/nprot.2015.128
20. Zhong H, Xuan L, Wang D, Zhou J, Li Y, Jiang Q (2017) Generation of a co-culture cell micropattern model to simulate lung cancer bone metastasis for anti-cancer drug evaluation. RSC Adv 7(35):21837–21847. https://doi.org/10.1039/C7RA01868A
21. Li AP, Bode C, Sakai Y (2004) A novel in vitro system, the integrated discrete multiple organ cell culture (IdMOC) system, for the evaluation of human drug toxicity: comparative

cytotoxicity of tamoxifen towards normal human cells from five major organs and MCF-7 adenocarcinoma breast. Chem Biol Interact 150(1):129–136. https://doi.org/10.1016/j.cbi.2004.09.010
22. van Midwoud PM, Groothuis GMM, Merema MT, Verpoorte E (2010) Microfluidic biochip for the perifusion of precision-cut rat liver slices for metabolism and toxicology studies. Biotechnol Bioeng 105(1):184–194. https://doi.org/10.1002/bit.22516
23. Sin A, Chin KC, Jamil MF, Kostov Y, Rao G, Shuler ML (2008) The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors. Biotechnol Prog 20(1):338–345. https://doi.org/10.1021/bp034077d
24. Viravaidya K, Shuler ML (2008) Incorporation of 3T3-L1 cells to mimic bioaccumulation in a microscale cell culture analog device for toxicity studies. Biotechnol Prog 20(2):590–597. https://doi.org/10.1021/bp034238d
25. Tatosian DA, Shuler ML (2009) A novel system for evaluation of drug mixtures for potential efficacy in treating multidrug resistant cancers. Biotechnol Bioeng 103(1):187–198. https://doi.org/10.1002/bit.22219
26. Mahler GJ, Esch MB, Glahn RP, Shuler ML (2009) Characterization of a gastrointestinal tract microscale cell culture analog used to predict drug toxicity. Biotechnol Bioeng 104(1):193–205. https://doi.org/10.1002/bit.22366
27. Sung JH, Kam C, Shuler ML (2010) A microfluidic device for a pharmacokinetic–pharmacodynamic (PK–PD) model on a chip. Lab Chip 10(4):446. https://doi.org/10.1039/b917763a
28. Oleaga C, Bernabini C, Smith AST et al (2016) Multi-organ toxicity demonstration in a functional human in vitro system composed of four organs. Sci Rep 6(1):20030. https://doi.org/10.1038/srep20030
29. Choe A, Ha SK, Choi I, Choi N, Sung JH (2017) Microfluidic gut-liver chip for reproducing the first pass metabolism. Biomed Microdevices 19(1):4. https://doi.org/10.1007/s10544-016-0143-2
30. Miller PG, Shuler ML (2016) Design and demonstration of a pumpless 14 compartment microphysiological system. Biotechnol Bioeng 113(10):2213–2227. https://doi.org/10.1002/bit.25989
31. Wnorowski A, Yang H, Wu JC (2019) Progress, obstacles, and limitations in the use of stem cells in organ-on-a-chip models. Adv Drug Deliv Rev 140:3–11. https://doi.org/10.1016/j.addr.2018.06.001
32. Yang Q, Lian Q, Xu F (2017) Perspective: fabrication of integrated organ-on-a-chip via bioprinting. Biomicrofluidics 11(3):031301. https://doi.org/10.1063/1.4982945
33. Esch MB, Mahler GJ (2019) Body-on-a-chip systems: design, fabrication, and applications. In: Microfluidic cell culture systems. Elsevier, Amsterdam, pp 323–350. https://doi.org/10.1016/B978-0-12-813671-3.00011-6
34. Wang K, Man K, Liu J et al (2020) Microphysiological systems: design, fabrication, and applications. ACS Biomater Sci Eng 6(6):3231–3257. https://doi.org/10.1021/acsbiomaterials.9b01667
35. Derby B (2012) Printing and prototyping of tissues and scaffolds. Science 338(6109):921–926. https://doi.org/10.1126/science.1226340
36. Jang J, Yi HG, Cho DW (2016) 3D printed tissue models: present and future. ACS Biomater Sci Eng 2(10):1722–1731. https://doi.org/10.1021/acsbiomaterials.6b00129
37. Ho CMB, Ng SH, Li KHH, Yoon YJ (2015) 3D printed microfluidics for biological applications. Lab Chip 15(18):3627–3637. https://doi.org/10.1039/C5LC00685F
38. Boland TW, Wilson WC, Xu T (2003) Ink-jet printing of viable cells. 7,051,654
39. Ringeisen BR, Othon CM, Barron JA, Young D, Spargo BJ (2006) Jet-based methods to print living cells. Biotechnol J 1(9):930–948. https://doi.org/10.1002/biot.200600058
40. Tumarkin E, Tzadu L, Csaszar E et al (2011) High-throughput combinatorial cell co-culture using microfluidics. Integr Biol 3(6):653. https://doi.org/10.1039/c1ib00002k

41. Lee VK, Kim DY, Ngo H et al (2014) Creating perfused functional vascular channels using 3D bio-printing technology. Biomaterials 35(28):8092–8102. https://doi.org/10.1016/j.biomaterials.2014.05.083
42. Mittal R, Woo FW, Castro CS et al (2019) Organ-on-chip models: implications in drug discovery and clinical applications. J Cell Physiol 234(6):8352–8380. https://doi.org/10.1002/jcp.27729
43. Catros S, Fricain JC, Guillotin B et al (2011) Laser-assisted bioprinting for creating on-demand patterns of human osteoprogenitor cells and nano-hydroxyapatite. Biofabrication 3(2):025001. https://doi.org/10.1088/1758-5082/3/2/025001
44. Jana S, Lerman A (2015) Bioprinting a cardiac valve. Biotechnol Adv 33(8):1503–1521. https://doi.org/10.1016/j.biotechadv.2015.07.006
45. Guillemot F, Souquet A, Catros S, Guillotin B (2010) Laser-assisted cell printing: principle, physical parameters versus cell fate and perspectives in tissue engineering. Nanomedicine 5(3):507–515. https://doi.org/10.2217/nnm.10.14
46. Guillemot F, Souquet A, Catros S et al (2010) High-throughput laser printing of cells and biomaterials for tissue engineering. Acta Biomater 6(7):2494–2500. https://doi.org/10.1016/j.actbio.2009.09.029
47. Barron JA, Wu P, Ladouceur HD, Ringeisen BR (2004) Biological laser printing: a novel technique for creating heterogeneous 3-dimensional cell patterns. Biomed Microdevices 6(2):139–147. https://doi.org/10.1023/B:BMMD.0000031751.67267.9f
48. Ringeisen BR, Kim H, Barron JA et al (2004) Laser printing of pluripotent embryonal carcinoma cells. Tissue Eng 10(3–4):483–491. https://doi.org/10.1089/107632704323061843
49. Melchels FPW, Domingos MAN, Klein TJ, Malda J, Bartolo PJ, Hutmacher DW (2012) Additive manufacturing of tissues and organs. Prog Polym Sci 37(8):1079–1104. https://doi.org/10.1016/j.progpolymsci.2011.11.007
50. Chang R, Nam J, Sun W (2008) Effects of dispensing pressure and nozzle diameter on cell survival from solid freeform fabrication–based direct cell writing. Tissue Eng Part A 14(1):41–48. https://doi.org/10.1089/ten.a.2007.0004
51. Kačarević Ž, Rider P, Alkildani S et al (2018) An introduction to 3D bioprinting: possibilities, challenges and future aspects. Materials 11(11):2199. https://doi.org/10.3390/ma11112199
52. Hull CW (1984) Apparatus for production of three-dimensional objects by stereolithography. US4575330A
53. Avci H, Doğan Güzel F, Erol S, Akpek A (2018) Recent advances in organ-on-a-chip technologies and future challenges: a review. Turk J Chem 42(3):587–610. https://doi.org/10.3906/kim-1611-35
54. Mandrycky C, Wang Z, Kim K, Kim DH (2016) 3D bioprinting for engineering complex tissues. Biotechnol Adv 34(4):422–434. https://doi.org/10.1016/j.biotechadv.2015.12.011
55. Tumbleston JR, Shirvanyants D, Ermoshkin N et al (2015) Continuous liquid interface production of 3D objects. Science 347(6228):1349–1352. https://doi.org/10.1126/science.aaa2397
56. Jain A, Barrile R, van der Meer A et al (2018) Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics. Clin Pharmacol Therap 103(2):332–340. https://doi.org/10.1002/cpt.742
57. Zhang R, Larsen NB (2017) Stereolithographic hydrogel printing of 3D culture chips with biofunctionalized complex 3D perfusion networks. Lab Chip 17(24):4273–4282. https://doi.org/10.1039/C7LC00926G
58. Ong LJY, Islam A, DasGupta R, Iyer NG, Leo HL, Toh YC (2017) A 3D printed microfluidic perfusion device for multicellular spheroid cultures. Biofabrication 9(4):045005. https://doi.org/10.1088/1758-5090/aa8858
59. Kim Y, Castro K, Bhattacharjee N, Folch A (2018) Digital manufacturing of selective porous barriers in microchannels using multi-material stereolithography. Micromachines 9(3):125. https://doi.org/10.3390/mi9030125

60. Gross BC, Erkal JL, Lockwood SY, Chen C, Spence DM (2014) Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences. Anal Chem 86(7): 3240–3253. https://doi.org/10.1021/ac403397r
61. Gale B, Jafek A, Lambert C et al (2018) A review of current methods in microfluidic device fabrication and future commercialization prospects. Inventions 3(3):60. https://doi.org/10.3390/inventions3030060
62. Gröger M, Dinger J, Kiehntopf M, Peters FT, Rauen U, Mosig AS (2018) Preservation of cell structure, metabolism, and biotransformation activity of liver-on-chip organ models by hypothermic storage. Adv Healthc Mater 7(2):1700616. https://doi.org/10.1002/adhm.201700616
63. Wu J, Gu M (2011) Microfluidic sensing: state of the art fabrication and detection techniques. J Biomed Opt 16(8):080901. https://doi.org/10.1117/1.3607430
64. Szydzik C, Gavela AF, Herranz S et al (2017) An automated optofluidic biosensor platform combining interferometric sensors and injection moulded microfluidics. Lab Chip 17(16): 2793–2804. https://doi.org/10.1039/C7LC00524E
65. Weibel DB, DiLuzio WR, Whitesides GM (2007) Microfabrication meets microbiology. Nat Rev Microbiol 5(3):209–218. https://doi.org/10.1038/nrmicro1616
66. Whitesides GM, Stroock AD (2001) Flexible methods for microfluidics. Phys Today 54(6): 42–48. https://doi.org/10.1063/1.1387591
67. Pasman T, Grijpma D, Stamatialis D, Poot A (2018) Flat and microstructured polymeric membranes in organs-on-chips. J R Soc Interface 15(144):20180351. https://doi.org/10.1098/rsif.2018.0351
68. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE (2010) Reconstituting organ-level lung functions on a chip. Science 328(5986):1662–1668. https://doi.org/10.1126/science.1188302
69. Bavli D, Prill S, Ezra E et al (2016) Real-time monitoring of metabolic function in liver-on-chip microdevices tracks the dynamics of mitochondrial dysfunction. Proc Natl Acad Sci U S A 113(16):E2231–E2240. https://doi.org/10.1073/pnas.1522556113
70. Gupta N, Liu JR, Patel B, Solomon DE, Vaidya B, Gupta V (2016) Microfluidics-based 3D cell culture models: utility in novel drug discovery and delivery research. Bioeng Transl Med 1(1):63–81. https://doi.org/10.1002/btm2.10013
71. Mathur A, Loskill P, Shao K et al (2015) Human iPSC-based cardiac microphysiological system for drug screening applications. Sci Rep 5(1):8883. https://doi.org/10.1038/srep08883
72. Polini A, Prodanov L, Bhise NS, Manoharan V, Dokmeci MR, Khademhosseini A (2014) Organs-on-a-chip: a new tool for drug discovery. Expert Opin Drug Discov 9(4):335–352. https://doi.org/10.1517/17460441.2014.886562
73. Liu Q, Zhang Z, Liu Y et al (2018) Cancer cells growing on perfused 3D collagen model produced higher reactive oxygen species level and were more resistant to cisplatin compared to the 2D model. J Appl Biomater Funct Mater 16(3):144–150. https://doi.org/10.1177/2280800018764763
74. Gozalpour E, Fenner KS (2018) Current state of in vitro cell-based renal models. Curr Drug Metab 19(4):310–326. https://doi.org/10.2174/1389200219666180119115133
75. Jain A, Barrile R, van der Meer A et al (2018) Primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics. Clin Pharmacol Ther 103(2): 332–340. https://doi.org/10.1002/cpt.742
76. Sung JH, Yu J, Luo D, Shuler ML, March JC (2011) Microscale 3-D hydrogel scaffold for biomimetic gastrointestinal (GI) tract model. Lab Chip 11(3):389–392. https://doi.org/10.1039/C0LC00273A
77. Gao D, Liu H, Lin JM, Wang Y, Jiang Y (2013) Characterization of drug permeability in Caco-2 monolayers by mass spectrometry on a membrane-based microfluidic device. Lab Chip 13(5):978. https://doi.org/10.1039/c2lc41215b
78. von Martels JZH, Sadaghian Sadabad M, Bourgonje AR et al (2017) The role of gut microbiota in health and disease: in vitro modeling of host-microbe interactions at the

aerobe-anaerobe interphase of the human gut. Anaerobe 44:3–12. https://doi.org/10.1016/j.anaerobe.2017.01.001
79. Kramer JA, Sagartz JE, Morris DL (2007) The application of discovery toxicology and pathology towards the design of safer pharmaceutical lead candidates. Nat Rev Drug Discov 6(8):636–649. https://doi.org/10.1038/nrd2378
80. Dubois-Deruy E, Peugnet V, Turkieh A, Pinet F (2020) Oxidative stress in cardiovascular diseases. Antioxidants 9(9):864. https://doi.org/10.3390/antiox9090864
81. Klauke N, Smith G, Cooper JM (2007) Microfluidic systems to examine intercellular coupling of pairs of cardiac myocytes. Lab Chip 7(6):731. https://doi.org/10.1039/b706175g
82. Kitsara M, Kontziampasis D, Agbulut O, Chen Y (2019) Heart on a chip: micro-nanofabrication and microfluidics steering the future of cardiac tissue engineering. Microelectron Eng 203–204:44–62. https://doi.org/10.1016/j.mee.2018.11.001
83. Martewicz S, Michielin F, Serena E, Zambon A, Mongillo M, Elvassore N (2012) Reversible alteration of calcium dynamics in cardiomyocytes during acute hypoxia transient in a microfluidic platform. Integr Biol 4(2):153–164. https://doi.org/10.1039/C1IB00087J
84. Agarwal A, Goss JA, Cho A, McCain ML, Parker KK (2013) Microfluidic heart on a chip for higher throughput pharmacological studies. Lab Chip 13(18):3599. https://doi.org/10.1039/c3lc50350j
85. Wang X, Lee J, Ali M, Kim J, Lacerda CMR (2017) Phenotype transformation of aortic valve interstitial cells due to applied shear stresses within a microfluidic chip. Ann Biomed Eng 45(10):2269–2280. https://doi.org/10.1007/s10439-017-1871-z
86. Marsano A, Conficconi C, Lemme M et al (2016) Beating heart on a chip: a novel microfluidic platform to generate functional 3D cardiac microtissues. Lab Chip 16(3):599–610. https://doi.org/10.1039/C5LC01356A
87. Sidorov VY, Samson PC, Sidorova TN, Davidson JM, Lim CC, Wikswo JP (2017) I-Wire Heart-on-a-Chip I: three-dimensional cardiac tissue constructs for physiology and pharmacology. Acta Biomater 48:68–78. https://doi.org/10.1016/j.actbio.2016.11.009
88. Liu H, Bolonduro OA, Hu N et al (2020) Heart-on-a-chip model with integrated extra- and intracellular bioelectronics for monitoring cardiac electrophysiology under acute hypoxia. Nano Lett 20(4):2585–2593. https://doi.org/10.1021/acs.nanolett.0c00076
89. Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC (2012) Skin anti-aging strategies. Dermato-Endocrinol 4(3):308–319. https://doi.org/10.4161/derm.22804
90. Tiwari V, Wilson DM (2019) DNA damage and associated DNA repair defects in disease and premature aging. Am J Hum Genet 105(2):237–257. https://doi.org/10.1016/j.ajhg.2019.06.005
91. Makrantonaki E, Zouboulis CC (2007) Characteristics and pathomechanisms of endogenously aged skin. Dermatology 214(4):352–360. https://doi.org/10.1159/000100890
92. Wen H, Gao X, Qin J (2014) Probing the anti-aging role of polydatin in Caenorhabditis elegans on a chip. Integr Biol 6(1):35–43. https://doi.org/10.1039/C3IB40191J
93. Chen Y, Mao P, Snijders AM, Wang D (2018) Senescence chips for ultrahigh-throughput isolation and removal of senescent cells. Aging Cell 17(2):e12722. https://doi.org/10.1111/acel.12722
94. Subia B, Dey T, Sharma S, Kundu SC (2015) Target specific delivery of anticancer drug in silk fibroin based 3D distribution model of bone–breast cancer cells. ACS Appl Mater Interfaces 7(4):2269–2279. https://doi.org/10.1021/am506094c
95. Jo Y, Choi N, Kim K, Koo HJ, Choi J, Kim HN (2018) Chemoresistance of cancer cells: requirements of tumor microenvironment-mimicking *in vitro* models in anti-cancer drug development. Theranostics 8(19):5259–5275. https://doi.org/10.7150/thno.29098
96. Fang Y, Eglen RM (2017) Three-dimensional cell cultures in drug discovery and development. SLAS Discov 22(5):456–472. https://doi.org/10.1177/1087057117696795
97. Festing S, Wilkinson R (2007) The ethics of animal research. EMBO Rep 8(6):526–530. https://doi.org/10.1038/sj.embor.7400993

98. Zhang YS, Zhang YN, Zhang W (2017) Cancer-on-a-chip systems at the frontier of nanomedicine. Drug Discov Today 22(9):1392–1399. https://doi.org/10.1016/j.drudis.2017.03.011
99. van Tellingen O, Yetkin-Arik B, de Gooijer MC, Wesseling P, Wurdinger T, de Vries HE (2015) Overcoming the blood–brain tumor barrier for effective glioblastoma treatment. Drug Resist Updat 19:1–12. https://doi.org/10.1016/j.drup.2015.02.002
100. Oliver CR, Westerhof TM, Castro MG, Merajver SD (2020) Quantifying the brain metastatic tumor micro-environment using an organ-on-a chip 3D model, machine learning, and confocal tomography. J Vis Exp (162). https://doi.org/10.3791/61654
101. Silvani G, Basirun C, Wu H et al (2021) A 3D-bioprinted vascularized glioblastoma-on-a-chip for studying the impact of simulated microgravity as a novel pre-clinical approach in brain tumor therapy. Adv Therap 4(11):2100106. https://doi.org/10.1002/adtp.202100106
102. Sun YS, Zhao Z, Yang ZN et al (2017) Risk factors and preventions of breast cancer. Int J Biol Sci 13(11):1387–1397. https://doi.org/10.7150/ijbs.21635
103. Choi Y, Hyun E, Seo J et al (2015) A microengineered pathophysiological model of early-stage breast cancer. Lab Chip 15(16):3350–3357. https://doi.org/10.1039/C5LC00514K
104. Zheng Y, Sun Y, Yu X et al (2016) Angiogenesis in liquid tumors: an in vitro assay for leukemic-cell-induced bone marrow angiogenesis. Adv Healthc Mater 5(9):1014–1024. https://doi.org/10.1002/adhm.201501007
105. Zhang YS, Aleman J, Shin SR et al (2017) Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proc Natl Acad Sci U S A 114(12):E2293–E2302. https://doi.org/10.1073/pnas.1612906114
106. Caballero D, Kaushik S, Correlo VM, Oliveira JM, Reis RL, Kundu SC (2017) Organ-on-chip models of cancer metastasis for future personalized medicine: from chip to the patient. Biomaterials 149:98–115. https://doi.org/10.1016/j.biomaterials.2017.10.005
107. Berthier E, Young EWK, Beebe D (2012) Engineers are from PDMS-land, biologists are from polystyrenia. Lab Chip 12(7):1224. https://doi.org/10.1039/c2lc20982a
108. Neužil P, Giselbrecht S, Länge K, Huang TJ, Manz A (2012) Revisiting lab-on-a-chip technology for drug discovery. Nat Rev Drug Discov 11(8):620–632. https://doi.org/10.1038/nrd3799
109. Zhu J (2020) Application of organ-on-chip in drug discovery. J Biosci Med 08(03):119–134. https://doi.org/10.4236/jbm.2020.83011

Multiorgans-on-a-Chip for Personalized Medicine

13

Pooja Kaushik, Medha Kaushik, Sharon Jacob, and Suhel Parvez

Abstract

Microfluidic devices with regulated, dynamic microenvironments in which grown cells exhibit activities that mimic organ-level physiology are known as organs on chips. Multiorgan-on-a-chip devices have the potential to revolutionize how human health research is carried out. In theory, they can be "personalized" for reflection of individual physiology by including blood samples, primary human tissue, and cells derived from induced pluripotent stem cell-derived cells based on personal health data. The personalized nature of such systems, along with physiologically relevant readouts, opens up new possibilities for person-specific pharmacological efficacy and safety assessments, as well as customized disease preventive and treatment methods. In the field of medicine, personalized medicine is a trendy topic. Personalized medicine is a theory for rational therapeutics as well as a practice to individualize health interventions (e.g., drugs, food, vaccines) using biomarkers. It is often presented with the motto "the right drug, for the right patient, at the right dose, and at the right time." In this chapter, we highlight the necessities and characteristics of several organ-on-a-chip models available today and how they are used in personalized medicine. Multiorgan-on-a-chip model has been detailed for specific use in personalized medicine therapy.

Keywords

Organoids · Organ-on-chip · Personalized medicine · Targeted therapy · Therapeutic interventions

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13.1 Introduction

The latest research from the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2019 presents troubling questions about global health's trajectory in the twenty-first century. On the one hand, the news appears to be positive. The world's population's health is continuously improving. From 67.2 years in 2000 to 73.5 years in 2019, the global life expectancy at birth has grown. In 202 of the 204 countries and territories, healthy life expectancy has grown with the help of modern medicine. Precision medicine and personalized medicine are the latest [1, 2].

Through a wide range of data, personalized and precision medicine aims to provide a platform for effective health management. Longitudinal tracking of healthy individuals to better understand the transition from non-diseased to diseased states; more precisely identifying individuals at risk for disease; and tailoring treatments based on diverse and growing data sets from both individual trials and population-based studies are all potential inputs for advancing precision medicine [3]. Personalized medicine (PM) has been the recent trend in medical research with the main goal to find the right drug for the right patient at the right time. As a result, PM stands for "the treatment of a patient's condition or disposition by applying the most up-to-date molecular knowledge in order to get the optimum medical outcome for that individual." PM is considered as the foundation of global health [4].

Human physiology is the study of the human body's functioning and organ systems. This is extremely important to our understanding of body dysfunction and etiology, and so is intimately related to medicine, medication development, and toxicology. In vivo investigations on humans or model organisms are the most relevant and direct methods for researching human physiology. Many lower-level components, including tissues, cells, proteins, and genes, interact and adapt to support bodily processes. As a result, revealing the underlying mechanisms of physiological phenomena alone through in vivo studies is difficult. Traditional two-dimensional (2D) cell culture techniques have served as a vital platform for life science research for decades [5]. The functionalities of diverse cells are examined using 2D systems by growing cells or cell products. However, 2D systems frequently require verification in in vivo animal models because they fail to effectively represent the physiological manifestations of living tissues/organs, intra-organ interactions, and microenvironmental factors. Animal research frequently fails to mimic human tests due to species differences, and the use of animals as drug testing models has come under fire due to both expensive costs and ethical concerns. In preclinical testing, a poor description of the human tissue milieu can lead to erroneous estimates of the overall effects of tissue function. Organ-on-a-chip (OOC) was created to address these flaws by expanding the number of physiological model systems available (Fig. 13.1). The use of OOC as a future replacement technology for experimental animal models has been proposed [7, 8].

Most OOC models, however, are focused on a single cell type or tissue and lack a systemic dimension as well as cross-organ communication. Multiple organs have been modeled in a single device as a multiorgan platform, which is a huge recent development (Fig. 13.2). Hence, this chapter discusses the recent advances from

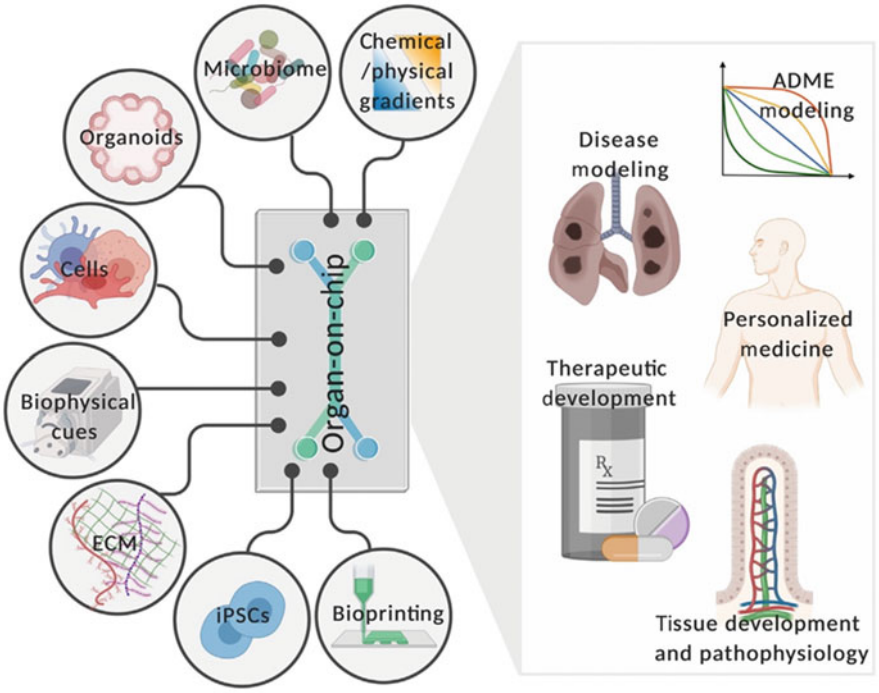

Fig. 13.1 Schematic representation of organ-on-a-chip systems. (*Adapted from* [6])

organ-on-a-chip technology with specific emphasis on multiorgan on a chip and its role in personalized medicine.

13.2 Personalized Medicine

The prescription of a specific therapeutic approach for an individual, based on pharmacogenomics information, is referred to as personalized medicine, which is a developing branch of medicine. It is now widely accepted that the underlying heterogeneity of many disease processes necessitates tailoring or "personalization" of strategies for treating, monitoring, or preventing a disease in an individual based on their unique biochemical, physiological, environmental exposure, and behavioral profile. Furthermore, personalized medicine classifies seemingly clinically homogeneous individuals into subpopulations with distinct clinical prognoses or treatment responses in practice [4, 10]. Personalized medicine is not the same as precision medicine. Precision medicine makes use of enormous amounts of population-level data to assist select the best treatment for a specific patient. Precision medicine differs from traditional medicine in that it elucidates how population-derived genomic, proteomic, or wider biomarker profiles might collectively decide an individual's treatment course. From diagnosis through therapy, the patient is treated

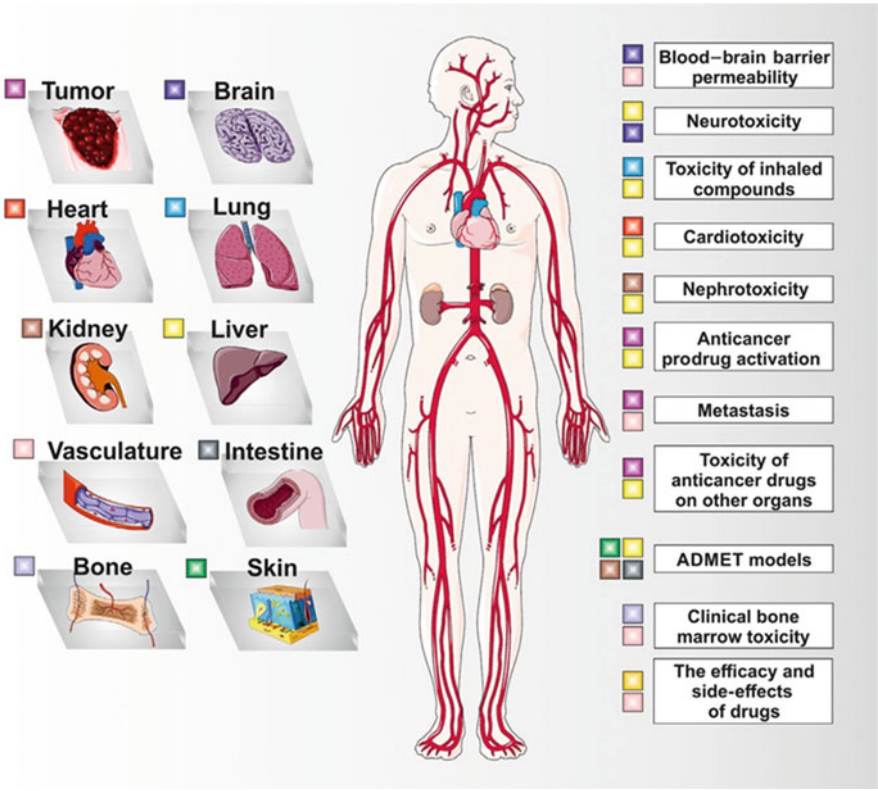

Fig. 13.2 Various multi-organ-on-a-chip combinations as a model of human physiology and pathophysiology in different biomedical applications. (*Adapted from* [9])

as an individual in personalized medicine, which clearly has some overlaps with precision medicine in execution, but does not fundamentally rely on huge data sets or population-based approaches to redefine disease, unlike precision medicine [3].

Side effects aren't the only source of inter-individual variability during drug treatment. Drug's intended therapeutic effects vary a lot from patient to patient and population to demographic. An examination of medications in important therapeutic areas such as Alzheimer's disease, cancer, and analgesics found that, on average, only around half of patients respond to drugs, while the other half do not. If drug safety and therapeutic effects were predictable and did not vary from person to person, personalized medicine would not be required, which is not the case. Most medications have side effects, some of which can be significant and fatal, and only a small percentage of patients have the anticipated therapeutic effects. The first step toward personalized medicine and rational treatments is to understand the mechanisms behind individual variability in drug safety and efficacy [11].

Traditional pharmacotherapies are used by the human body to treat disease in two ways. The pharmacokinetic activity, that is, the ADME (Absorption, Distribution,

Metabolism, and Excretion), of a drug is often controlled by a unique set of genes (e.g., drug metabolizing enzymes) that may contain naturally occurring genetic variants (or "polymorphisms") that influence their function and thus how the body ultimately deals with a drug. When a medicine enters the body, its pharmacodynamic qualities describe how it interacts with its target (usually a gene or a protein encoded by a gene) to produce an effect [4]. Many early examples of tailored therapeutics were linked to drug pharmacokinetics that were influenced by genetics. Warfarin is a commonly prescribed blood thinner that, if not used correctly, can result in a potentially fatal adverse drug reaction. Warfarin is metabolized in part by the gene CYP2C9 and targets the VKORC1 gene. Variation in the pharmacologic and pharmacokinetic features of Warfarin is caused by naturally occurring genetic variation in both the VKORC1 and CYP2C9 genes, resulting in variation in individual reactions to the drug. As a result, the US Food and Drug Administration has suggested that warfarin doses take into account an individual's genotype [4]. Primaquine (PQ) is another excellent example of a medicine that should only be given to those with a specific genetic profile. In malaria-endemic areas of the world, PQ was used to manage the disease with some success. Military medics in the past noticed that some of the troops they treated for malaria who were given the medicine grew jaundiced and anemic, eventually displaying symptoms of what would later be known as "acute hemolytic anemia (AHA)." It was eventually discovered that the people who developed AHA after taking PQ had glucose-6-phosphatase dehydrogenase (G6PD) gene variations. As a result, current PQ clinical practice requires genotyping of individual patients to see if they have important G6PD gene variations that may inhibit PQ treatment [4, 10].

Instead of developing a drug and then identifying factors that reduce its efficacy or side effects through observational studies on people who were given the drug, as was the case with warfarin and PQ, researchers are now attempting to identify, for example, genetic profiles possessed by patients and then crafting therapies that target those profiles specifically. Immunotherapies, which are a new class of cancer medicines, are classic example of such therapy. One sort of immunotherapy takes advantage of "neo-antigens," which are potentially unique combinations of genetic abnormalities that occur in a cancer patient's tumor cells and can typically elicit an immune response if appropriately recognized by the host's immune cells [4, 11].

Several technologies, such as molecular diagnostic tools, have been critical in the development of personalized medications in order to find unique "biological markers" that have resulted in the optimal medical treatment for each patient. Personalized therapy is a costly technology, but it is commercially practical because it reduces drug development costs by shortening the research cycle by the application of pharmacogenomics [10]. Because different people respond differently to the same medicine, pharmacogenomics is critical for personalized medicine. The goal of pharmacogenomics is to identify genetic variations that may have an impact on drug efficacy and toxicity. Overall, the domains span a broad range of topics, including basic drug discovery, the genetic origins of pharmacokinetics and pharmacodynamics, innovative drug development, patient genetic assessment, and clinical patient administration. Finally, the goal of pharmacogenomics is to predict a patient's

genetic response to a medicine in order to provide the best-possible medical treatment. It will be feasible to improve the efficacy of medicines and reduce the incidence of adverse side effects by predicting an individual's pharmacological response [12]. Personalized medicine should be labeled as an outcome if it will be most faithful and strong in specific subpopulations or if it will need to be directed in extremely high dosages in a specific subpopulation. Pharmacogenomics can help choose between responders and nonresponders to drugs, prevent adverse effects, and optimize drug dosage. Drug labels may only incorporate genomic biomarker knowledge and may describe: medication disclosure and clinical response variability, risk of side effects, genotype-specific mediation, structures of medication action, polymorphic medication target and nature genes [3, 11, 12].

13.2.1 Prerequisites of Personalized Medicine

The ability to select the right patient for the drug, customize dosage regimens, noninvasively monitor drug movement in the body, archive information on drug response for unique patient attributes, and develop simulation models to guide the design and interpretation of clinical trials in the context of drug safety are all part of the personalized medicine model. To that goal, personalized medicine must meet the following four requirements:

- Biomarkers
  A critical prerequisite is the development of illness-specific biomarkers for determining the stage of disease at the time of first diagnosis and assessing disease progression thereafter. Developing physiologically sensitive drug carriers is also critical for tailoring drug release profiles to specific conditions and maximizing therapeutic benefits. Research towards finding and validating disease-specific biomarkers would be immensely beneficial in managing disease complexity as well as enabling earlier disease detection and thus medication therapy initiation. However, at an advanced stage, the patient is unlikely to respond to treatment in a significant way [13].
- Drug Monitoring
  Today, constant input to the patient and physician on the progress of pharmacological therapy is uncommon. Drug monitoring, on the other hand, will be an important part of individualized treatment. Indeed, establishing drug-specific point-of-care (POC) diagnostics to quantify treatment response is a logical progression from biomarker-based diagnostic screening kits. This will provide helpful information to the patient and the healthcare professionals who are responsible for his or her treatment, especially when the condition has no evident indications or symptoms. Furthermore, if the medication response signal can be digitalized and sent via the web to a server for data warehousing, it may be able to search for efficacy and toxicity patterns to make therapeutic modifications. Companion noninvasive molecular imaging would be an equally useful tool for mapping medication distribution in the body, especially target access. This information,

together with the establishment of innovative biomaterials and nanotechnology, would be invaluable in guiding the development of targeted medication delivery systems. Incorporating these technologies earlier in the medication development cycle will raise the likelihood of success by optimizing therapeutic advantages and minimizing side effects, as well as increasing the economic return by expanding the range of therapeutic indications. The plethora of clinical and pharmacokinetic data can be kept and used to test disease simulation models in the future. This is the essence of informatics, a potentially beneficial tool for recognizing at-risk populations in order to monitor adverse medication reactions [14].

- Smart Drug-Delivery System
  It is necessary to develop a new generation of medication delivery devices that are significantly different from fixed-dose tablets and capsules. These innovative technologies, which range from microneedles to a modified inkjet printer, are intended to allow patients, particularly the elderly, greater flexibility in dispensing multiple medicine dosages. Customizing drug dosage can be based on the patient's genetics and lifestyle, as well as co-existing conditions (and thus additional drugs) [14].
- Reformation of Regulatory Policy
  To deal with the influx of new drug product applications focusing on subpopulations, the regulatory infrastructure and philosophy must be upgraded, including creating standards for products with unmet public health requirements and improving safety and health through informatics. Regulatory authorities may need to develop incentives to acknowledge the sponsor's ongoing leadership in innovation, quality assurance, and socially responsible in order to make regulatory decisions on time. It's critical to have a well-designed demonstration project that will throw light on the wisdom of a de facto honor system of regulatory control.

  Regulatory agencies must be ready to shift from a reactive to a proactive mode of operation, as well as embrace a continual mode of invention and improvement. Regulatory bodies are supposed to identify problem areas in drug production, champion and reward breakthroughs in drug development and manufacturing, and ensure that drug products are manufactured in accordance with international standards [14].

13.2.2 Emerging Technologies in Personalized Medicine

- Precision Therapeutics
  CRISPR (clustered regularly interspaced short palindromic repeats) and zinc finger nucleases (ZFN) are gene-editing techniques that can change particular components of a genome, bringing up new avenues for precision gene repair in diseases ranging from cancer to HIV. CRISPR's democratization of gene editing promises to generate massive volumes of data that will hopefully shed light on novel disease links and pathways that have never been linked to a specific disease

before. The use of gene editing in patients has just lately become commonplace [15].

Precision medicine's foundations, molecularly targeted and antibody treatments, have resulted in a wide range of authorized medications and new ways of patient-specific therapeutic ranking using mutation databases. The use of CRISPR with an enzyme called CRISPR-associated protein-9 nuclease (Cas9) to fix genetic abnormalities in disorders like Duchenne muscular dystrophy (DMD) is one of the most recent genome- and proteome-guided therapeutic methods in preclinical research. New delivery techniques, like as nanoparticles, can also be used to boost the efficacy of CRISPR. In cells and animals, a recent preclinical study employing CRISPR/Cas9 and a lipid nanoparticle successfully switched off genes that cause elevated cholesterol levels. CRISPR-Cas9 delivery through gold nanoparticles was utilized to improve the efficacy of repairing a DNA mutation linked to DMD. Novel nanocarrier techniques for RNA interference are being used in addition to CRISPR-mediated guide RNA-based treatment [16].

- Wearable and Implantable Sensors
  For indications ranging from pulmonary hypertension to cancer therapy, frequent and comprehensive readout monitoring is required. Wearable technology will play a crucial role in facilitating tailored therapy in this regard. Blood oxygenation and heart rate measures have also been produced in wearable, battery-free devices. Noninvasive measurements of sweat, tears, skin interstitial fluid, and saliva are linked to blood concentrations of these analytes using soft electronics. These systems can be used to detect glucose and monitor cystic fibrosis without the drawbacks of fingerstick testing. Pulse measurements have also been developed using microfluidics-embedded polymer wristbands. Silicone microfiber tubes with electrodes for blood pressure, blood vessel stiffness, and heart rate monitoring have been developed, allowing for noninvasive diagnosis and monitoring of atherosclerosis, venous ulcers, and possible incorporation into bandages for a variety of applications. This type of noninvasive, multiplexed monitoring could eventually lead to the discovery of individualized biomarker signatures [3].
- Bioengineering for Personalized Medicine
  The "one-size-fits-all" paradigm in medicine is fading. We're starting to realize that patients with the same disease can react differently to anything from medications to biomaterials, and it's important to learn more about this and treat each patient as an individual. Engineers can help make individualized medicine a reality in clinics. For instance, consider biomaterials. Researchers discovered that a dextran–dendrimer composite behaves differently as an adhesive in different organs and in different settings. These findings show that most biomaterial applications are not universal, and that the illness indication and organ environment play a role in the design of materials that will only be present in the body for a short time. Biomaterials can affect cell destiny, survival, and growth in diverse ways, but there aren't many high-throughput methods for finding the best material composition for a specific application. To discover the optimum polymer carrier for islet transplantation, one method is to use a

combination of small and large animal models. Various extracellular matrix (ECM) formulations (different organs, different processing methods) were studied to see how they affected stem cell development, cancer cell proliferation, and cell death. New platforms for determining the best biomaterial formulation could increase tailored biocompatibility and therapeutic outcomes. Individualized drug screening and toxicology studies are being investigated using OOC platforms. For example, a microfluidics-based model of the human intestine was recently constructed, enabling for the monitoring of interactions between the gut microbiome, bacteria, and immune cells in the complex gut milieu. The capacity to reproduce organ-scale complexity utilizing platforms like our gut model opens the door to drug development and toxicity investigations involving pharmacokinetics, absorption, and drug metabolism study. These abilities could potentially be the basis for developing individualized treatments. As customized medicine advances, new areas of development have included the use of nondrug-based platforms like digital therapy to treat problems including mild cognitive impairment, substance misuse, mental health, and attention deficit hyperactivity disorder, among others [17, 18].

- Artificial Intelligence, Machine Learning, and Personalized Treatment
  The difficulty to determine drug doses that are best suited for an individual patient has been a key roadblock to the optimization of any targeted therapy, especially as dosing requirements frequently alter during treatment. Given the almost unlimited dose parameter space that exists in combination therapy, this is much more difficult. The concept of determining population-optimized drug-dose ratios faces the same problem. To address this issue, traditional patient dosing and medication development have relied on identifying the drugs to be provided first, then conducting dose escalation trials to determine the maximum tolerated dose (MTD). Drug-synergy prediction modeling and other in silico technologies have been used to improve the likelihood of good treatment results [19].

  Furthermore, instead of predictive drug discovery, synergy modeling, or monotherapy or combination therapy selection, artificial intelligence (AI) and machine learning (ML)-related platforms are now capable of deterministically detecting drug administration parameters in a continuous fashion. More particularly, by reconciling the almost endless parameter space formed by these elements, the adoption of novel AI-based platforms is accurately revealing the crucial link between drug dose and drug selection. AI is also being used to improve diagnostic imaging skills in order to better guide patient-specific treatment. Furthermore, AI is being used in the field of therapeutics to concurrently discover the best medications and doses for maximum combination therapy efficacy and safety, even from very vast pools of candidate compounds.

  The use of AI is projected to grow as new platforms for patient-specific testing and therapy become available. OOC technology, single-cell interrogation platforms, and sophisticated diagnostic modalities, for example, could be appropriate testbeds. OOC technologies, in particular, are capable of mimicking complex functions in both healthy and pathological systems, spanning from the gut to

placental transport and pulmonary function. Recent breakthroughs in OOC development have paved the way for drug screening applications [20].

13.3 Organs-on-a-Chip: Addressing Unmet Needs

Overall, using OOCs that represent the variety of human genetics, physiology, and disease, the dual incentives of de-risking drug development and personalizing patient treatment can be fulfilled. In 2003, the first attempts to combine cell culture with microfluidics were made, resulting in the progenitors to today's OOCs. Today's OOC platforms, which use microfluidics and tri-dimensional cell culture to build micro-sized human tissues/organs, can assist speed up drug research by bridging the gap between animal models, cell culture, and clinical investigations in terms of medication safety and efficacy. Clinical trials, the most expensive and risky step of drug development, could be revolutionized by the quantitative and mechanistic data acquired utilizing these human OOC models. Instead of treating future patients as a group, using patient-specific cells allows researchers to capture the essential variances that arise from genetic diversity, ethnicity, gender, and age. Furthermore, corporate acceptance of OOCs will aid present attempts to minimize, refine, and eventually replace animal models (3Rs) with more ethical alternatives [21].

OOC is a type of microfluidic device fabricated with the silicon-based organic polymer polydimethylsiloxane (PDMS) using the standard soft lithography technique; as a result, the chip has a compact size and microchannels to precisely pattern cells and manipulate various fluidic and chemical parameters, such as flow rate, pressure, oxygen, and pH, allowing for controllable culture conditions. This represents the microstructural and functional properties of human tissues and organs in vivo, allowing for more effective and precise medical, biological, and pharmacological research. Despite the potential for a revolution in the pharmaceutical business, the total impact of the OOC approach is unknown, due to significant obstacles in the transition from fundamental research to preclinical integration of this platform into the drug development pipeline [21].

13.4 Design Concept and Key Components of Organs-on-a-Chip

13.4.1 Design Concept

External and internal cell conditions must be controlled in culture systems. External parameters may be controlled and physiological conditions can be precisely simulated using OOC in conjunction with micromachining and cell biology. On the chip, dynamic mechanical stress, fluid shear, and concentration gradients are all necessary. To accurately portray physiological processes, cell patterning should also be achieved.

Fluid shear force: Microfluidics allows for dynamic cell culture by micro-pump perfusion, allowing for more efficient nutrient administration and waste removal. Static culture is less comparable to in vivo circumstances than the dynamic environment in which cells are placed. Organ polarity is also induced by fluid shear stress. Importantly, OOC activates cell surface molecules and associated signaling cascades, exerting required physical pressure on endothelial cells' regular biological functions. Biological analyses at the single organ level are also possible because of the inclusion of fluid in the OOC device. For organization-specific settings, the OOC system summarizes flow using a simple "rocker" on a chip fluid motion or a more complex programmable "pulsa-tile" format, all of which are arranged in a single loop [22, 23].

Concentration gradient: The fluid behaves largely as a laminar flow at the micro-scale, resulting in a stable gradient of biological molecules that can be regulated both geographically and temporally. Angiogenesis, invasion, and migration are examples of biological events that use biochemical signals driven by concentration gradients. Microfluidics simulate complicated physiological processes in the human body by changing flow velocity and channel shape with microvalves and micropumps to generate stable three-dimensional (3D) biochemical concentration gradients [24, 25].

Dynamic mechanical stress: Blood pressure, lung pressure, and bone pressure are all normal day-to-day organ pressures. Mechanically stressed tissues such as skeletal muscle, bone, cartilage, and blood vessels are maintained in part by these pressures. Microfluidics allow for the creation of periodic mechanical stresses using elastic porous membranes. During physiological processes, mechanical stimulation is thought to be a significant factor of differentiation [26].

Cell patterning: To develop an effective whole-body relationship, the human body requires a sophisticated and orderly arrangement of numerous cells. For the design of in vitro physiological models with complex geometries, microfluidics governs cell patterning. Cell patterning on the chip is aided by surface changes, templates, and 3D printing. By allowing the production of hydrogel scaffolds with complicated pathways, the 3D printing method allows for multiscale cell patterning. The advantage of 3D printing is that it allows for user-defined digital masks to give versatility in cell patterns, which is crucial for in vitro cellular microenvironment rebuilding. Using controlled topological manipulations, Li et al. established strategies to produce quick heterotypic cell patterning on glass chips. On a glass chip, this technology combines polyvinyl acetate coating, carbon dioxide laser ablation, and continuous cell seeding procedures. Controlled epithelial–mesenchymal interactions are possible with this approach. Glass chips can also be patterned with mesenchymal cells with comparable capabilities. This approach can be used to investigate and evaluate the cutaneous epithelial–mesenchymal interaction on a wide scale, and it can also be used to study the patterning of other cells [27, 28].

13.4.2 Key Components

(1) Microfluidics, (2) living cell tissues, (3) stimulation or drug administration, and (4) sensing are the four fundamental components of the OOC. The microfluidic component involves a system of culture fluid input and waste liquid outflow throughout the culture process, as well as the use of microfluidics to deliver target cells to a pre-designated area. Miniaturization, integration, and automation are common characteristics of this component. In 2D or 3D systems, the living cell tissue component refers to components that spatially align a specific cell type. Biocompatible materials, like as hydrogels, are commonly used to build 3D arrangements. These materials have the ability to resist mechanical damage and can be used to create three-dimensional arrangements. Although 3D tissue structure more accurately reflects the in vivo condition than 2D models, living cells in organ tissues are still largely cultured in 2D due to technological and financial limitations, as well as the assembly of extracellular matrix and the pre-setting and development of vasculature. To imitate the physiological milieu, which supports micro-tissue maturation and function, physical or chemical cues are necessary for some tissues. Electrical stimulation, for example, can aid in the growth of cardiac tissue. Drug screening procedures can be derived from a variety of signal sources. An embedded sensing output component or a transparent chip-based visual function evaluation system can be used as the sensing component for detecting and compiling data. Peel et al. imaged multicellular OOCs with automated techniques, yielding precise cell phenotypes and statistical models for measurements. Kane et al. created a cell system to track cells in a three-dimensional microfluidic environment. Through quality control, these experiments used time-lapse imaging microscopy to detect cellular electrical activity. Microsensor-mediated sensing of the metabolic state at characteristic places in the system is required to describe and access a relevant human-on-chip cell model [28, 29].

13.5 Organs-on-a-Chip Models

Advanced in vitro models of multicellular tissue complexes or functioning organ units developed in microfabricated cell culture devices are referred to as OOC. OOC is a word that is interchangeable with tissue-on-a-chip and micro-physiological systems. Constructing these customized model systems is an engineering design approach that follows the reductionist principle and takes advantage of the precision of cell culture microfabrication technologies [30].

13.5.1 Single Organ-on-a-Chip Systems

Researchers have been attempting to use various microfluidic devices and lab-on-a-chip systems to enable regulated and organotypic cell culture for in vitro biochemical and pharmacological investigations since the early 2000s, which sparked the idea of

an OOC system. In 2010, the Harvard Medical School's Ingber group published a lung-on-a-chip model based on Huh's early Takayama work, which drew widespread attention from the biology and engineering worlds and was hailed as a watershed moment in the development of OOC. Since then, numerous single-organ chips have been successfully developed for investigating disease progression and analyzing adverse drug reactions, including liver chips, kidney chips, pancreas chips, heart chips, intestine and gut chips, blood–brain barrier (BBB) chips, and bone and bone marrow chips. At the preclinical research stage, these single-organ chip assays can assist identify essential biological mechanisms as well as test drug efficacy and toxicity in target organs, providing a trustworthy reference for clinical trials [21].

13.5.1.1 Brain-on-a-Chip

The brain is the nervous system's central organ. The skull, as well as other physical structures such as the BBB and the meninges, a layer of membranes that covers the CNS, protect it. Inflammation, on the other hand, can allow viruses to enter the brain and compromise these protective barriers. All physiological functions are controlled by the brain, which also integrates information from the environment. It is made up of a number of structures, the majority of which are made up of two cell populations: neurons and glial cells. Afferent and efferent routes, which transfer information by chemical or electrical signals, make up the neural circuitry. They are necessary for the transmission of information that takes advantage of currents traveling along the axon membrane to synapses. Glial cells provide neurons with protection, food, and structural support. Astrocytes, microglia, oligodendrocytes, and ependymal cells are among them [31]. Researchers have spent a lot of time studying the structure and functions of the brain, but modeling the complete brain while taking into account the varied cell populations and surface dimensions is a huge problem. 3D cultures have aided in the development of novel medicines and improved clinical translation. Bioprinting has been shown to be capable of fabricating structures containing glial cells, neurons, and stem cells in the setting of 3D neural tissue models. OOCs are vital tools for supporting the life and growth of brain tissue, assessing cell migration, the direction of axonal extension, transport, and signal transmission, thanks to their interconnecting chambers and dynamic perfusion [32].

Brain organoids are being utilized to learn more about how the Zika virus (ZIKV) is linked to congenital microcephaly and to evaluate prospective anti-ZIKV medication options. In this case, the brain organoid is very beneficial since it lets researchers to study the human brain as it develops [33]. Spheroids (as well as organoids generated from stem cells) are potent 3D biological platforms for studying neuronal growth, drug transport, and neuronal disease etiology. They rely on the ability of tiny cell aggregates to construct polarized floating structures in vitro, without the use of any established pattern or foreign material, that are similar to in vivo tissues. For example, one study proposed a microfluidic chip for the interstitial perfusion of neurospheroids with flow parameters similar to those in the brain (approximately 0.1–0.3 L/min) and used it as an in vitro model of Alzheimer's disease to test the toxicity of Aβ [34]. Wang et al. have also created a microfluidic chip for obtaining

human-induced pluripotent stem cell (hiPS)-derived brain organoids in order to explore neurodevelopmental problems during the early stages of pregnancy. They created a PDMS-based device with five separate channels using traditional soft lithography. Their findings show that organs on a chip provide a regulated microenvironment for brain organoids' effective development, maturation, and extended growth [35].

Kilic et al. employed organs on a chip to increase culture duration, offer a regulated and reliable microenvironment for hPSC differentiation into neuronal and astroglial cells, and investigate cell movement in response to chemotactic cue gradients. The integration of the BBB and the brain in a single OOC is a step forward in the development of a fully miniaturized drug screening and toxicity model [36]. For example, Koo et al. used a device called OrganoPlate R. (MIMETAS, The Netherlands). They used a collagen hydrogel to fill the brain compartment with N2a neuroblastoma cells, C8D1A immortalized astrocytes, and BV-2 immortalized microglia. They defined the blood–brain barrier (BBB) compartment after gelation by depositing bEnd.3 EC against the hydrogel [37]. Because excitability is a crucial property of brain cells, combining organ-on-a-chip technology with electrodes is critical for stimulating and reading the burst-firing power and frequency rate within the constructs. Soscia et al., for example, describe removable inserts that allow neurons from various brain areas (e.g., primary rodent hippocampal and cortical neurons) to be deposited into designated portions of a commercial microelectrode array (MEA) without the necessity of physical or chemical barriers [38].

13.5.2 Lung-on-a-Chip

Both alveolar tissue and arteries are important targets in the study of drug discovery for the lungs. Improvements have been made to the architecture of the lung organ-on-a-chip model in order to advance drug discovery and better understand the physiological roles of certain proteins, relationships, and disorders. In comparison to 2D plating-type architectures and structures that lack crucial ventilation dynamics, these technologies provide a more realistic approach to simulating the 3D environment in which in vivo organs live.

As the lung fills with air, the respiratory regions expand and contract cyclically to maximize the surface area available for gas exchange. When the alveolus (the space between the vascular endothelium and the pulmonary epithelium separated only by a basement membrane) is considered the smallest functional unit of the lung, the cyclic expansion can be mimicked by stretching the gas exchange surface mechanically. Huh et al. [39] created a lung-on-a-chip model by dividing the chip into areas separated by 10 m PDMS membranes with an extracellular matrix (ECM) using soft lithography [39]. Alveolar epithelial cells were found in the top PDMS sections, while human pulmonary microvascular endothelial cells were found in the bottom parts, simulating the alveolar–capillary barrier. Under vacuum, the membrane architectures were changed to imitate the expansion and contraction of the alveoli

during respiration. Neutrophils were used to inject inflammatory stimuli into the system, which were then transferred through the fluid channels.

The administration of interleukin-2 (IL-2) resulted in a pathological model of pulmonary edema [39]. This demonstrates how the OOC models can be used to improve current in vivo experiments. The resulting lung on a chip was the first of its type, demonstrating organ-level behavior successfully. A similar design of a lung OOC chamber with liquid phase flow was optimized using mathematical modeling, resulting in regulated gas concentrations within both the gas and liquid sides of the alveolus–capillary interface as well as a way to quantify changes in gas transfer between the compartments. In 2015, Stucki et al. published a lung chip that approximated the parenchyma of the lungs. The system comprised an alveolar barrier and a 3D cyclic strain that simulated breathing, making it the first elastic membrane expansion model to do so [40]. Blume et al. created 3D airway culture models that used fluid and media exchange to imitate pulmonary interstitial flow. This made it possible to do more in-depth physiological research on the epithelial barrier. This model combines many chambers for enhanced integration and uses a stent with a permeable filter as a single tissue culture chamber. Pressure can be applied to the alveoli and associated capillaries in the lung on a chip, giving a shear flow profile while emulating lung gas–liquid interfaces and respiratory dilation through the microfluidic system. This accurately replicates the lung environment [41]. To test their applicability as a physiological model, Humayun et al. cultivated airway epithelial and smooth muscle cells on opposite sides of a hydrogel membrane. As a physiological model of chronic lung disease, the system was integrated with microenvironment cues and toxin exposure [42]. Yang et al. developed an electrospinning nanofiber membrane made of poly(lactic-co-glycolic acid) (PLGA) as a chip matrix for cell scavenging. According to the researchers, the system's ease of use makes it suitable for lung tumor precision therapy and tissue engineering techniques [43].

A microfluidic device was developed by Benam et al. [44] that connects a differentiated mucociliary bronchiolar epithelium exposed to the environment with a basement epithelium that experiences fluid flow similar to blood flow. This research shows how to create a tiny airway, or air–blood barrier, for studying physiological and pathophysiological systems, the parameters of which can be changed depending on how well the condition is understood. Benam's model overcomes the existing major problem with airway mucosa, which is recapitulating the impacts of a fully interactive immune system and endothelial tissues that are subjected to shear stress from blood flow into the imitated airway's function. This improved lung-on-a-chip model enables for more research into lung disorders that involve the immune system, such as asthma and the comorbidities of chronic obstructive pulmonary disease (COPD) [44].

According to Tan et al. [45], the engineered airway organoid is a system that is important for the study of diseases involving airway cell–cell interactions and that these organoids have excellent branching morphogenesis—a gateway into grafting implantable airway tissues for cell-based therapy. Finally, when these organoids were implanted, it was observed that while they did not grow well in vivo, they did

help the underlying cells regenerate and promote vascularization in the area, suggesting that they could be used to treat some disorders [33].

Nuclear phosphorylated catenin (p-β-cateninY^{489}) is a characteristic finding in premature baby lung cells with bronchopulmonary dysplasia (BPD) and in fetal lung tissue that is missing in term births. Immunohistochemistry for p-β-cateninY^{489} was used to test this in an organoid model of human fetal lung (HFL) cells. A new approach for selectively inducing hiPSC into either proximal or distal airway epithelium for the study of lung function and disorders was recently developed. Implantable respiratory support devices can be made from lung tissue organ chips. Peng et al. created lung assist devices (LAD) to allow for increased gas exchange in the placenta during respiratory failure in preterm newborns. Large-diameter channels were created in the umbilical arteries and veins, enabling significant extracorporeal blood supply to the LAD. Because clinical trials for umbilical vasodilation thresholds were unethical, this provides added value. This was the first study to comprehensively quantify umbilical artery damage caused by catheter expansion [46]. To increase gas exchange, Dabaghi et al. used microfabrication for microfluidic blood oxygenators with double-sided gas supply. In comparison to single-sided devices, oxygen consumption increased by 343% [47]. Xu et al. employed a microfluidic chip platform to evaluate several chemotherapeutic medicines in a microfluidic chip platform that mimicked the microenvironment of lung cancer with cancer cell lines and primary cancer cells. Another recent study used a "small airway-on-a-chip" device to simulate asthma. Therapeutics were examined using human asthmatic and chronic obstructive pulmonary disease airway models, and the chip model accurately replicated in vivo responses to a similar medication [48].

13.5.3 Heart-on-a-Chip

The heart and the blood vessels are the two main organs of the mammalian circulatory system that is responsible for the pumping of blood throughout the body. This system is autorhythmic and its function is modulated by the endocrine and nervous system. The heart pumps blood in response to the excitation of the cardiac conduction system that is the network of nodes, cells, and signals responsible for controlling the heartbeat and can be considered the smallest functional unit of the heart muscles [49–52]. The development of OOC models of the heart and the circulatory system helps to understand the functioning of the heart, the transport of nutrients, oxygen, and even drug molecules throughout the body. Cardian OOC models also help in studying the physiology and mechanism of cardiovascular diseases which is the leading cause of death worldwide. Another aspect of the design of the OOC model of the heart is the toxicity assessment of various drugs because after toxicity, cardiac toxicity is the leading cause for the failures of drug candidates that mainly manifests as changes in function like arrhythmia. With the help of the OOC technology, it is even possible to produce replicating functional cardiovascular organs that show vascular phenomenon like endothelial permeability, vasoregulation, and platelet thrombus formation [9].

Throughout the years there have been many different types for the design of OOC model of the heart. In one case, a cardiac biowire bioreactor was microfabricated using human embryonic stem cells (hESCs) and primary neonatal rat cardiomyocytes for pharmaceutical testing. In this model the beating (pulse) of the cardiac biowires could be reduced using nitric acid in the medium. Another heart-on-a-chip methodology was used to make an accurately controllable physiological mechanical stimulation and this helps to rebuild stimulative physiological conditions for the testing and production of cardiac patches that can be used to replace injured cardiac tissues. Yet another example of an OOC model is a device that generates a cardiac-like flow in a closed culture system. There are also many cardiac OOC design considerations that are made with human-induced pluripotent stem cells (hiPMS), which incorporates different types supporting cells like fibroblasts and endothelial cells; electromechanical stimulations; real-time observation of cardiac contractility and electrophysiology. These help in enhancing maturation and enabling physiological drug responses [49].

The organ-on-a-chip model of the heart can also be used to study the complex process of multiorgan system interactions. For instance, we can use it to study the interaction of the cardiac system and the effect of the presence of a liver-on-a-chip system when interacted with a drug like epinephrine or propranolol. The effects of epinephrine and propranolol were first tested using an independent cardiac-only system followed by its testing when a cardiac chip and liver chip were integrated to form a dual-organoid system. The treatment with propranolol resulted in a small, but significant decrease in the beating rate of the cardiac system. But, in the dual-organoid system, there was no change in the beating rate that shows that the drug was metabolized. Treatment with epinephrine resulted in an increase in the beating rate in the cardiac system. But, in the dual-organoid system there was no change in the epinephrine induced beat rate [51].

13.5.4 Spleen-on-a-Chip

A lymphoid organ, the spleen, is a small soft organ located in the upper left side of the abdomen, behind the rib cage and stomach. It is a critical component of the immune system. It functions as a filter, removing viruses, germs, worn or damaged red blood cells (RBCs), and other pathogens from the circulation. It is divided into two primary regions, the red pulp and the white pulp, by an interface known as the marginal zone [53]. Despite its importance in immunity, few attempts to replicate the spleen in vitro have been attempted, possibly because survival without one is possible. Rigat-Brugarolas and colleagues created a microfluidic system that simulates the physical features and hydrodynamic forces of the spleen's basic functional unit, the splenon. The device has two channels, one for fast fluid flow and one for slow fluid flow, which is similar to red pulp microcirculation. Blood passes via a pillar matrix in the slow channel, which increases the hematocrit of the blood, and then through constricted channels, forcing the cells to deform. The degree of distortion changes with cell healthiness and can be used to distinguish healthy

from diseased cells. Healthy and malaria-infected RBCs were used to test the device's filtration ability [54]. Despite proving the ability of a spleen on a chip to distinguish between healthy and diseased cells, there is still room for improvement, particularly in terms of how damaged RBCs and other pathogens are removed or destroyed by macrophages. Ingber and colleagues created a microfluidic device that acts as an external filtration system similar to a hemodialysis machine, simulating both the blood filtering and cleansing functions of the spleen [55]. Multiple Gram-negative and Gram-positive bacteria, fungi, and endotoxins were successfully removed from entire human blood using the spleen-on-a-chip system. Although this spleen-on-a-chip device is designed to treat sepsis, it can also be used in drug development and discovery, as well as personalized medicine, by speeding up the process of pathogen identification and drug susceptibility determination, especially because the modified protein can bind to both live and dead pathogens [56].

13.5.5 Liver-on-a-Chip

The liver is the major site of metabolism in the human body and is involved in multiple functions like regulation of glycogen of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and the detoxification of the body. The microstructure of the liver is complex with many different cells interacting with one another. The hepatocytes make up 70–85% of the liver's mass and it is the main functional parenchymal cell of the organ. The main functional unit of the liver consists of the hepatic lobules or the liver sinusoid that helps in metabolism. Thus, hepatocytes act as the representative model when developing artificial liver in vitro. For studying the liver functions, we see that there are lot of limitation in the reproduction of the in vivo environment using the conventional cell culture systems. Culture of hepatocytes leads to decreased functionality over time [33, 57]. The development of the organ-on-a-chip technique has allowed for a continuous medium flow with exchange of oxygen and waste materials. This creates a more physiological environment in stimulating the expression of detoxifying genes. The liver-on-a-chip system was first developed for pharmacokinetic/pharmacodynamic applications as liver toxicity and liver metabolism are the leading causes of drug candidate failures [51].

Multiple liver-on-a-chip models based on hepatocytes were developed in which the cells were cultured using different approaches like spheroids, sandwich gels, porous scaffolds, or encapsulation in natural or synthetic hydrogels. Another report shows that rat hepatocytes were isolated and it was encapsulated in polyethylene glycol diacrylate. This model was able to maintain liver functions for over 50 days and it responded to prototypic small molecules and drug–drug interactions. Liver organ on a chip that contains hepatocytes and fibroblasts arranged in different ways has also been developed. Liver sinusoid organ-on-chips have been developed that incorporate microchannels containing densely packed hepatocytes joined to an endothelial barrier or by using co-cultures accompanied by a fluid flow (liver blood circulation). Studies have tried to mimic the structure of liver sinusoids that

consists of a cell culture area, an endothelial-like barrier, and a nutrient transport channel. Liver-on-a-chip models allow for the analysis of the first pass effect and analyze various cytochrome P450 enzymes like CYP3A4 and CYP2C19 [33, 51, 57].

There have been many advancements in liver-on-a-chip device designs to study hepatic functions and liver-based multiorgan communication like [58].

1. The bioprinted liver-on-a-chip construct made using HepG2/C3A cell line organized in spheroids suspended in hydrogel for studying drug screening applications.
2. The liver-on-a-chip model to study hepatitis B virus infection made using primary human hepatocytes co-cultured with Kupfer cells.
3. The microfluidic device to investigate nonalcoholic fatty liver disease made using the HepG2/C3A cell line.
4. The multiorgan chip model for the co-culture of gastrointestinal (GI) tract epithelium and three-dimensional primary liver. This was made using epithelial cell line Caco-2, primary hepatocytes, and primary nonparenchymal cells (NPCs).

The liver-on-a-chip model can also be used to study the pharmacokinetics and toxicity of drugs. Toxicity assays use the liver-chip and another target organ to form a multiorgan on a chip model. For example, combining liver and the small intestine allows to understand absorption. As such combining this model with other organs like endothelium, brain, pancreas, gut, heart, lung, muscle, kidney allows for accurate pharmacokinetic and pharmacodynamic analysis [9]. The liver-on-a-chip-based devices allow to mimic the native hepatic environment. They are able to host 3D cell constructs and can be perfused with a continuous flow of fresh medium, while allowing simultaneous real-time and noninvasive microscope monitoring.

13.5.6 Intestine-on-a-Chip

The intestine plays a very crucial role in the human digestive system, in absorption, as a barrier and also in the immune system. Many of the human diseases are also associated with the dysfunction of the intestine function like obesity, inflammatory bowel disease. The human intestine is a very complex organ with highly vascularized tissues, complex architecture, and different cell types that include enterocytes and endocrine cells. The intestine also possesses the enteric nervous system that governs the gastrointestinal functions. The intestine also undergoes a movement called peristalsis. Another feature of the intestine is the presence of a communal system called the gut microbiota [57]. The traditional methods of study like using the Ring and segment of rat's intestine or everted Sac were harvested directly from the animals. This was the reason that during the process of drug screening, even though more than 90% of the drug candidates passed the animal testing phase, they showed inadequate therapeutic effects and even proved toxic in humans due to species differentiation. Animal models were most commonly used to

study the intestine, but increase in animal welfare issues and the species differentiation between animals and humans were the two main limitations to its continued use to study the intestines [59].

Novel methods/devices to study the intestine include the Transwell chamber, the gut-on-a-chip model and multiorgan chip models. The gut OOC was developed from microfluidic devices mainly using Caco-2 cells, derived from colon cancer that can easily differentiate into intestines with villous structures and the tight junctions between cell mucus secretions. The use of Caco-2 cells is able to reproduce the basic structure and function of the intestinal organs. The induced pluripotent stem cells (iPSCs) can also be used in place of the Caco-2 cells to create the gut-on-a-chip model [57, 59, 60]. The development of the gut OOC models allows for the study of the absorption, metabolism, and transport of drugs delivered orally. They also enable to create the microenvironment for drug testing.

The gut microbiota is a natural source of metabolites, hormones, and toxins that regulate the gut physiology and also extra-intestinal organs like the liver and brain. The gut microbiota plays a key role in drug biotransformation, by directly or indirectly influencing drug absorption, toxicity and bioavailability. The human-microbial cross-talk model (HuMiX) is an effective model to recreate the complex structure and physiology of the intestinal epithelium and it also provides a tool for in vitro drug testing and the study of the effects of the intestinal microbiome on drug availability. For example, a nonpathogenic *Escherichia coli*, co-cultured with intestinal cells in a gut-on-a-chip model resulted in a good symbiotic relationship between the cells and the *E. coli* [33, 58, 60].

The OOC model also proves a template to study multiorgan interactions like the gut–liver interaction. Chip-based models of the liver and gut for studying the gut–liver interaction have been developed using various cells like hepatoma cell lines, hepatic microsomes, and primary hepatocytes. The combination of the gut and liver models helps to study the first-pass effect when there is a fluidic circuit. The gut and liver can be co-cultured in a microfluidic device and the drugs can be made to interact with the gut and liver cells. The resulting drugs and metabolites in the circulating media can be quantified. This interaction can also be used to study the nonalcoholic fatty liver disease (NALFD) [58, 60, 61].

Every year millions of people die due to colorectal cancer and according to the WHO it is the third leading cause of cancer-related deaths. The gut-on-a-chip models have proven useful in establishing living physiological environments to test anticancer drugs. The OOC model allows integration of multiple mini-organs in different microchambers, interconnected by microfluidic channels and this gives a unique platform to study the progression of multiorgan cancer metastasis via the circulatory system [62].

13.5.7 Kidney-on-a-Chip

The kidney is responsible for maintaining osmotic pressure and drug excretion. Nephrotoxicity causes irreversible renal filtration loss, emphasizing the importance

of drug screening methods. The glomerulus, renal capsule, and renal tubule are responsible for filtration and reabsorption in the nephrons. Microfluidics can mimic the fluid environment that supports tubular cell growth while also providing porous membrane support for cell polarity maintenance [21]. Jang et al. developed the first multilayered microfluidic device to imitate renal filtration using mouse kidney medullary collecting duct cells. In response to hormone stimulation, the device created a biomimetic environment that improved polarity of the inner medullary collecting duct by stimulating cytoskeletal remodeling and molecular transport. Human primary renal epithelial cells were cultured using the same microfluidic technology in 2013. These were the first studies of primary kidney cells in terms of toxicity. This device allows for direct visualization and quantitative analysis of a variety of biological processes in the intact kidney tubule in ways that aren't possible with traditional cell culture or animal models, and it could be useful for researching basic molecular mechanisms of kidney function and disease [63].

Importantly, the proximal convoluted tubule structure has been replicated in the bioprinting arm using renal fibroblasts, endothelial cells, and primary human renal proximal tubule epithelial cells expressing the protein transporters P-gp and SGLT2, as seen in vivo, as well as responding to nephrotoxic and nephroprotective substances. Using genetic markers, organoid models could help to better understand the prognosis of polycystic kidney disease (PKD). The advancement gained in the organ-on-a-chip technology arm was particularly outstanding. In a unique way, hiPSCs were converted into podocytes and co-cultured with renal parenchyma to generate a functioning glomerulus. In addition to this breakthrough, a promising clinical path was opened by incorporating a kidney-on-a-chip equipment into an existing kidney function detection unit. This point-of-care (POC) device could detect creatinine levels with a substantially lower sample size and a faster turnaround time. These technologies, combined with recent discoveries, lead to the conclusion that organ-on-a-chip models will progressively pave the way for more capable drug discovery and application in the future [21].

It was previously impossible to replicate the effect of podocytes on the glomerulus due to a lack of nephron-mimetic chip technology; instead, only the human proximal convoluted tubule could be replicated. These techniques were unable to successfully produce podocytes. The tissue–tissue contact of the glomerulus between podocyte and parenchyma, as shown by Musah et al. [64], could be seen. Selective differentiation of hiPSCs into a specific cell lineage has proven difficult; however, the exhibited strategy successfully phenotypic matured these cells in an intermediate mesoderm environment. In vivo, the glomerulus and podocytes are both derived from intermediate mesoderm. Drug studies could be done and generalized because co-cultured podocytes and glomerular parenchymal cells created a functioning glomerulus with an endothelialized apparatus. SIMPLE, a promising POC technology that includes a self-powered microfluidic pump, was combined into the well-known Creasensor and was able to detect creatinine levels in the pathophysiologic range for patients with chronic kidney disease (CKD). Improvements in the design of kidney-on-a-chip models have been created in

order to advance drug development and better understand the physiological roles of certain proteins and their interactions [26].

13.5.8 Female Reproductive Organ-on-a-Chip

The female reproductive system is made up of a set of interconnected organs that help with steroid hormone synthesis, oocyte formation, egg and sperm union, and fetal development. In order to guide ovulated oocytes, prepare for implantation, and nurture a fetus to develop as an autonomous organism, the organs of the female reproductive tract work as a cohesive system and are dynamically synchronized. The ovaries, fallopian tubes, uterus, and cervix are the primary organs of the female reproductive tract [65]. Several studies have reported the development of 3D micro-engineered devices for the reproduction of sections of the female reproductive system in the last several years, thanks to advances in microfluidics and organs-on-a-chip research. Modeling of the human placenta, uterus, and recreation of the 28-day menstrual cycle is one of them. Modeling pregnancy and its related problems have been central to a growing corpus of research aimed at exploring the possibilities of this technology for reproductive biology and medicine. OOC provides in vitro platforms for researching many aspects of human reproduction (Table 13.1) [66–68].

Pregnancy is a complicated, interconnected physiological process in which the female reproductive system goes through a series of complex, interdependent physiological events. Modeling these various stages of pregnancy is a critical goal in reproductive research with significant therapeutic implications. Elevated progesterone levels induce dynamic cellular and vascular changes in the endometrium during the luteal phase of the menstrual cycle, resulting in the creation of the decidua, a specialized mucosal layer on the uterine wall. The decidualization of maternal tissue, which serves to generate a receptive environment for embryo implantation, is a crucial first stage in pregnancy. For the study of decidualization, Gnecco et al. created a micro-engineered in vitro model of the human endometrium. The researchers developed a multilayered microdevice that allowed primary human endometrial fibroblasts and human umbilical vein endothelial cells (HUVECs) to co-culture on opposite sides of a thin membrane. Additionally, the group used this system's co-culture arrangement to look into vascular contributions to endometrial stromal cell decidualization [69, 70].

Blundell et al. established an in vitro micro-engineered cell culture system that co-cultivates human trophoblast cells and human placental villous endothelial cells under dynamic flow conditions to imitate the architecture of the human placental barrier. Upper and lower microchannels are divided by a thin semipermeable membrane in the model, which is a compartmentalized microfluidic system. The placental barrier's structural integrity was determined by examining the uniform development of cell–cell connections over the whole porous membrane. Furthermore, the trophoblasts cultivated in the upper chamber for 3 days had a dense coating of microvilli on their apical surface, which are typical membrane protrusions that function as a crucial regulator of placental transport. It was also able to modify the

Table 13.1 Summary of several OOC models (*Adapted from* [33])

Organ	Latest advance	Future directions
Heart	Ability to mimic Frank-Starling mechanics in cardiomyocytes Display proper auxotonic contractions	Lacking well-developed models that utilize adult phenotype cardiomyocytes for drug studies Limited generalization
Kidney	Recapitulate mature and functional podocytes in glomerulus allows for more selective filtering in models Mimic diabetes mellitus type II nephropathy at glomerulus	Produce more complete nephron-mimetic device Incorporate glomerulus with podocytes and proximal convoluted tubule
Lung	Biomechanical ventilation shown to increase resistance of certain lung cancers to traditional therapy and in models used by pharmaceutical companies for drug discovery	All-inclusive model including, inflammatory studies, immune system involvement, and biomechanical ventilation not produced to study cancer
Intestine–gut–stomach	Many host–microbe interactions with intestine organoid (*Salmonella*, *H. pylori*, *C. difficile*)	Unable to understand epithelial–mesenchymal interactions Lumen is an organoid issue
Liver	Bioprinting liver that maintains function for longer time than before Better opportunity to study drug metabolism	Combining bioprinting and microfluids to make multiorgans to understand first-pass effect better
Placenta	Successfully modeled drug transport using placenta-on-a-chip	Models using primary cells instead of cancer-derived cells
Brain	Can model early embryonic brain development Study teratogens Understand which drugs inhibit the Zika virus from entering developing embryonic brains	Standardization and reproducibility of brain organoids
Spleen	Device that mimics physical properties and hydrodynamic forces of splenon, the basic functional unit of spleen developed Device that mimics both blood filtering and cleansing function of spleen, acting as an external filtration system (analogous to the hemodialysis machine)	There is room for improvement specifically with regard to implementation of the removal or destructive process of damaged RBCs and of other pathogens by macrophages and dendritic cells Use can be extended to drug development discovery and personalized medicine by accelerating process of pathogen identification and drug susceptibility determination
Skin	Many models developed to aid process of drug development and testing varying in level of complexity, availability of vascular elements, types of cells incorporated Incorporation of vascularization into skin-on-a-chip models utilizing primary endothelial cells	Improvements in architecture like inclusion of fat or nerve tissues as lipophilic drug reservoir, immune cells, and nervous system Vascularized skin-on-a-chip models lack key immune cells such as dendritic cells and T-lymphocytes, undermining immunocompetence of skin

(continued)

Table 13.1 (continued)

Organ	Latest advance	Future directions
Multiorgan (miscellaneous)	Able to demonstrate differential toxicity of bleomycin (heart-only condition displayed nontoxicity) in a multiorgan construct of heart, lungs, and liver Postmetabolism, bleomycin showed cardiomyocyte toxicity by altering heart rate and eventually stopping the beating	Each organ treated and chemically matured as generic and whole organ Goal of integrating multiorgan system to personalize individual medical needs

soluble microenvironment of trophoblast cells in the maternal compartment using forskolin supplemented medium to stimulate their syncytialization utilizing this "placental-barrier" model [71].

Wei-Xuan et al. recently developed a microfluidic "uterus-on-a-chip" that can be utilized in in vitro fertilization-embryo transplantation (IVF-ET) procedures to mimic uterine activities like ovulation, insemination, and embryo growth. Two polydimethylsiloxane (PDMS) layers are separated by a porous polycarbonate (PC) membrane, which serves as a substrate for endometrial cell culture in the microfluidic "uterus-on-a-chip-chip." The top layer is a zigzag-shaped channel with a sequence of interlaced micro-sievers for oocyte capture, while the bottom layer is made up of four perfusion parallel channels with an array of micropillars on the bed to maintain the porous membrane. This microfluidic chip allows oocyte and endometrial cells to co-culture, simulating the shape and function of the uterus and aiding molecular passage over the membrane [67]. Recent research has revealed the ability to mimic, interrogate, and pharmacologically alter the pathophysiology of intractable illnesses of the human reproductive system utilizing micro-physiological systems. Preterm birth, ovarian cancer, and endometriosis are three key clinical difficulties in reproductive medicine, and a growing number of research is focusing on representational organ-chip models of pathophysiological mechanisms implicated in them [30].

13.5.9 Skin-on-a-Chip

The skin, which is made up of numerous layers of ectodermal tissue and is the biggest organ of the integumentary system, serves as the first line of defense against potentially dangerous external influences. Skin-on-a-chip (SoC) devices have been shown to improve skin analogues' barrier function, thicken the epidermis, and improve keratinocyte differentiation. However, when coupled to numerous OOC devices to form a human-on-a-chip for measuring system-wide effects, they become their most powerful application [72, 73]. By optimizing the static Transwell

assembly, great progress has been made in constructing microfluidic skin-on-a-chip models. In an air–liquid interface model, Alexander et al. created a skin-on-a-chip device capable of detecting both TEER and extracellular acidification rates. The scientists were able to detect the impact of sodium dodecyl sulfate in different co-cultures using this device [74]. Similarly, Sriram et al. used an upscaled technique to create skin analogues with little hydrogel shrinkage using a mass-producible four-layer platform based on the polymer polymethyl methacrylate (PMMA) [75]. Mori et al. created a skin-on-a-chip device with perfusable vascular channels and an air–liquid interface to more realistically replicate epidermal and dermal tissue. Lee and colleagues developed a perfused microfluidic model to show how fluid flow affects the survival of a vascularized 3D skin model with dermal and epidermal characteristics [76]. Alberti et al. also confirmed similar diffusion behaviors of caffeine, salicylic acid, and testosterone using a skin-on-a-chip platform, reducing sample variation by half when compared to an in vitro skin permeation assay based on a static Franz diffusion cell loaded with similar organotypic skin equivalents [77].

Wufuer et al. used a skin-on-a-chip model to induce TNF-α mediated edema in order to test dexamethasone as a therapy for epidermis and dermis swelling. This device, which consists of three stacked compartments separated by a porous PET membrane, assesses drug translocation by using an epidermal, dermal, and vascular layer. Another technological advancement is the incorporation of white adipose tissue into the hypodermis, as adipose tissue beneath the dermis can act as a reservoir for hydrophobic pharmacologically compounds [78]. Only the epidermis combined with vascularized full thickness skin, including adipose tissue, on a chip, will be able to provide a physiologically realistic and translatable alternative model for pharmacological and pharmacokinetic uptake research. In order to develop a miniature model that resembles physiological skin, future on-chip skin simulations will need to account for the immune system as well as the neurological system [72]. One of the most appealing applications of SoC is medicine and cosmetic research. Skin, as the body's outermost organ, provides a highly accessible route for transdermal and topical drug delivery, as well as serving as the final destination for many cosmetic items. In vitro/ex vivo monitoring of particle penetration across the epidermal barrier was pioneered by Lukács et al. Three functional cells make up the platform: a drug delivery compartment, a skin sample compartment, and a receptor compartment. The drug under investigation is kept in the drug delivery compartment, which is above the skin equivalent. To determine particle penetration, the contents of the receptor compartment at the basal skin layer are collected and evaluated. The platform was tested on rodent skin to see how well caffeine penetrated as a hydrophilic drug model [79].

13.5.10 Multiorgans-on-a-Chip

While single-organ chips focus on simulating individual organ functions, multiorgan chips that combine multiple organ units in a single chip, such as the gut compartment for drug absorption, the liver compartment for drug metabolism, and the kidney

compartment for drug elimination, have recently become popular to allow for more comprehensive studies (Fig. 13.3). More than 70% of drug attrition and withdrawal from the market is due to drug-induced toxicities in the liver, heart, kidney, and brain. Off-target interactions and excessive drug binding to toxicity-prone cells are common causes of adverse medication responses. Interconnected multitissue cell culture models are regarded a good tool to research the pharmacokinetic profiles of pharmacological molecules because poor pharmacokinetics and pharmacodynamics are significant contributors in medication failure (Fig. 13.4) [21, 72].

Van et al. were the first to use a microfluidic device to merge the liver and intestines. The chip worked with intestinal and liver slices, demonstrating its applicability to organ interactions such as bile acid production regulation [80]. This technique allowed for in vitro research and revealed information about organ–organ interactions. Pires de Mello et al. created a heart–liver–skin three-organ system to study the effects of acute and chronic drug exposure on both heart and liver functions. A four-organ chip was also designed for assessing the systemic toxicity of drug candidates, including sequentially connected gut, liver, skin, and kidney compartments and stable homeostasis across distinct organ compartments [81]. The effects of the anticancer drug 5-FU and the two pro-drugs of 5-FU (CAP and tegafur) in terms of intestinal absorption, hepatic metabolism, and growth inhibition in cancer and connective tissue were evaluated in a more advanced, pneumatic, pressure-driven, four-organ system composed of intestine, liver, cancer, and connective tissue models of multiorgans on chip [72].

The intricacy of the system grows as the number of organs on the chip grows, inevitably leading to unforeseen effects. To achieve a larger range of applications,

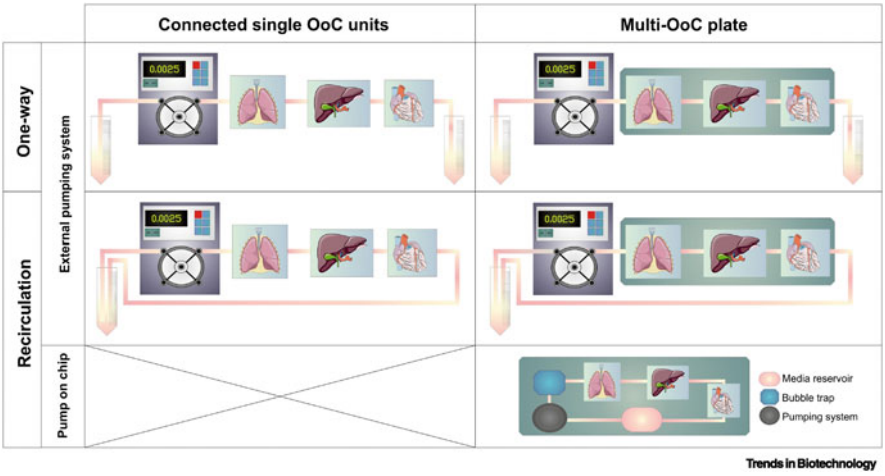

Fig. 13.3 Various strategies for establishing communication between different organ models in a multiorgan-on-a-chip (OOC) platform. (*Adapted from* [9])

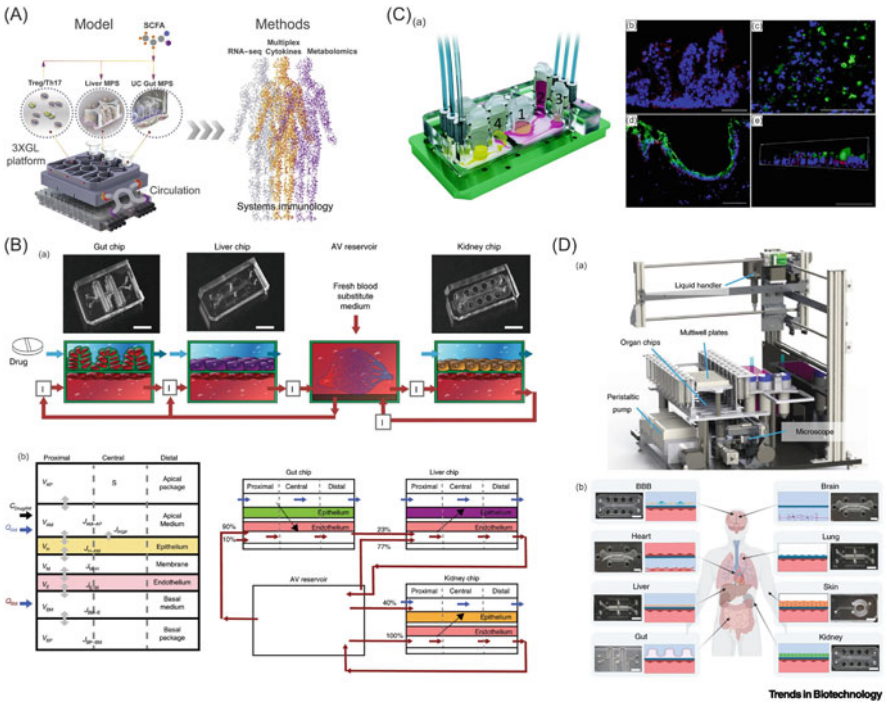

Fig. 13.4 Examples of multiorgan-chip systems, which enable disease modeling, analysis of drug pharmacokinetics (PK)/pharmacodynamics (PD) across tissues, and the creation of body-on-a-chip platforms. (*Adapted from* [6])

existing systems must be simplified. Satoh et al. described a multithroughput multiorgan-on-a-chip system built on a microplate-sized pneumatic pressure-driven medium circulation platform. For drug discovery, this system has the following advantages: simultaneous operation of multiple multiorgan culture units, microfluidic network design flexibility, a pipette-friendly liquid handling interface, and applicability to widely used experimental protocols and analytical methods in microplates. This multiorgan culture platform will be a useful tool for drug discovery research [82].

A more advanced version, labeled as "body-on-a-chip" or "human-on-a-chip," is now being developed to mimic the physiology of the full human body utilizing a single platform for medication pharmacokinetic and pharmacodynamic analysis. Miller and Shuler, for example, created a proof-of-concept 13-organ system using various cell lines to represent the main parenchymal organs and physiological barrier tissues of humans, demonstrating a physical framework for studying inter-organ commutation in response to drug challenges at the human level [83].

13.6 Multiorgans-on-a-Chip in Personalized Medicine

Through undesired side-effects in secondary tissues, the complicated ADME process impacts the destiny, distribution, efficacy (if applicable), and possible toxicity of exogenous chemicals (e.g., food, medications, additives, environmental contaminants). As a result, many diseases involving several organs, such as sepsis, osteoarthritis, gout, infertility, and neurodegenerative disorders, require systemic techniques to effectively simulate them. Similarly, detecting biomarkers in body fluids for diagnostic purposes requires interpreting this cross-organ communication. As previously described, multiple organs have been modeled in a single device as a multiorgan platform, which is a big recent development. To create multi-OOC platforms, two key approaches are being pursued: connecting single-OOC units and integrating many organs into one plate (multi-OOC plates) [9]. The multi-OOCs are recently being used for several applications in health sciences, such as toxicity screening, drug metabolism, pharmacokinetics, and pharmacodynamics studies (Fig. 13.5).

13.6.1 Toxicity Screening

Because toxicity is directly linked to liver metabolism, multi-OOC techniques for toxicity testing must include a liver model as well as at least one additional (target)

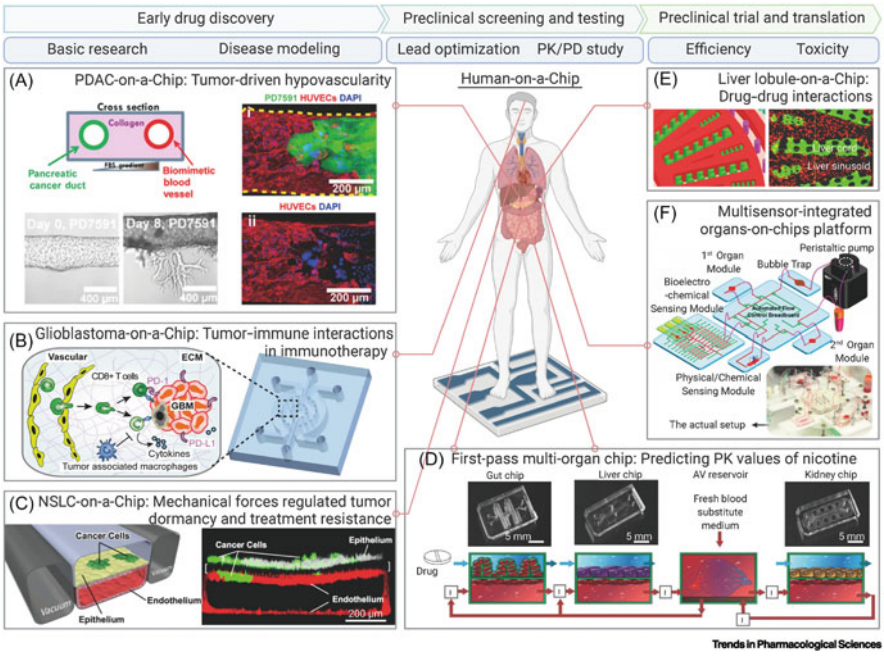

Fig. 13.5 Organ-on-a-chip platforms in preclinical drug development. (*Adapted from* [21])

organ. Human liver spheroids have been integrated with a 3D lung epithelial model, for example, to investigate the acute and chronic toxicity of inhaled aerosols or medicines. Alternatively, while evaluating the unfavorable side effects of a therapeutic therapy, both the target organ and the organ where side effects are expected, such as the kidney (nephrotoxicity) and the heart (cardiotoxicity), should be considered (cardiotoxicity) [84, 85].

13.6.2 Drug Metabolism

Prodrugs that only become biologically active after hepatic metabolism can also be tested using multi-OOC platforms that facilitate liver–target organ contact. Using spheroids co-cultured under flow conditions in a 96-well format and a self-adjusting modular Tetris-like microfluidic device, this bioactivation process was effectively recapitulated for cyclophosphamide targeting of colorectal cancer (TILE). Drug-induced skin sensitization was successfully predicted in vitro using a liver–immune system co-culture by modeling drug metabolism in 3D liver tissue and measuring immune cascade activation by the metabolites. The usefulness of multi-OOC models for evaluating systemic medication effects that involve several processes and organs is demonstrated in this example [82, 86].

13.6.3 Pharmacokinetics and Pharmacodynamics Studies

By definition, PK studies aimed at understanding and predicting the biological effects (therapeutic or harmful) of xenobiotics on the body need modeling of numerous organs and their interactions. A study demonstrated that the PK of orally delivered anticancer medicines could be evaluated by combining liver and lung with the small intestine. Other organs, such as the endothelium, brain, and testis, as well as the liver, pancreas, gut, lung, heart, muscle, brain, skin, kidney, and endometrial, were added to this multi-OOC platform, enabling for very accurate pharmacokinetic/pharmacodynamic (PK/PD) research [87].

13.6.4 Personalized Organs-on-Chips

Organs-on-chips can be personalized by engineering components of the physical, chemical, and biological microenvironment using patient samples and health data. Primary samples obtained from patients such as biopsies, surplus or waste tissue following surgery, and blood, stool, or urine samples are the most direct way to produce a personalized OOC [88].

Human blood samples from specific individuals have also been shown to be perfused via blood vessels-on-chips with “generic” vascular tissue while platelet aggregation and thrombosis in response to endothelial stimulation are monitored. Interestingly, blood samples from people taking antiplatelet drugs like aspirin and

clopidogrel revealed considerably less thrombosis patterns in activated blood vessels-on-chips than samples from control subjects, suggesting the ability to detect treatment response in individual patients. Decellularized extracellular matrix is another potential source of patient-derived primary material. Tumor-derived extracellular matrix, for example, has an impact on essential processes like cell proliferation and migration. There are currently no examples of extracellular matrices incorporated in organs-on-chips that are person- or patient-specific, but multiple examples have demonstrated how to controllably integrate extracellular matrices in organs-on-chips, such as through local hydrogel patterning or 3D-bioprinting [89, 90].

Transient expression of four transcription factors can re-program a patient's somatic cells (e.g., skin fibroblasts, blood-derived mononuclear cells, or urine-derived renal epithelial cells) to a pluripotent state. In theory, the resulting human-induced pluripotent stem cells (hiPSCs) can differentiate into any human tissue while remaining genetically identical to the original donor. Adult human stem cells, meantime, can be extracted from donor tissue and differentiated in the lab into the tissues from whence they come, with epidermal, intestinal, and gastric adult stem cells being notable examples. Both hiPSCs and adult stem cells provide researchers with an endless supply of patient-derived material due to their natural proliferation potential [91].

Controlled integration of biomarkers in organs-on-chips has been utilized to simulate patient characteristics in a variety of ways. Biomedical imaging is becoming more sophisticated, with a rise in modalities that can visualize not only the shape of a tissue or organ, but also its function in terms of blood flow, oxygenation, transporter characteristics, and so on. This vast repository of clinical information could theoretically be utilized to create personalized organs-on-chips with geometries, flow parameters, oxygen levels, and other culture factors derived from patient imaging data [88].

13.7 Conclusion

Given the fact that clinically meaningful inter-individual variation has been recognized and will continue to be identified, personalized medicine, or the practice of characterizing an individual patient on a number of levels (e.g., genomic, biochemical, and behavioral) that might shed light on their response to an intervention and then treating them accordingly, is a necessity. Modern biomedical technologies, such as DNA sequencing, proteomics, and wireless monitoring devices, have made it possible to identify this diversity, effectively revealing the need for some kind of personalization of therapy. OOCs are well-suited for capturing personal health-related parameters in a regulated system and producing functional read-outs that are directly linked to tissue- and organ-level physiology (Table 13.1). Despite the fact that tremendous efforts have improved the complexity, quality, and robustness of OOC models, recent initiatives are now taking this technology to the next level by developing multi-OOC platforms that aim to emulate entire biological processes that

are rarely limited to a single organ. Multi-OOC technology can simulate human physiology at the whole-organism level, with high accuracy and model complexity, as well as new opportunities in a variety of fields, all while supporting the "3Rs" (replacement, reduction, and refinement of animal models) and the personalized medicine paradigm.

13.8 Future Perspective

The future problems associated with personalized medicine will be to enhance not only the efficiency of how individuals are described, but also how individualized treatments are produced and vetted to demonstrate their utility. This is not to argue that interventions that work everywhere (i.e., typical single-agent "blockbuster" medications) should be overlooked if discovered, but it does mean that they may be difficult to find in the future. Maintaining the homeostasis of many organs and combining all of the critical cues, such as hormonal stimulation, the immune system, the lymph, the microbiome, and organ innervation and vascularization, that have been mostly ignored to date, remain major hurdles. Multimodal real-time analysis in multi-OOC platforms should be developed as a future step, involving the integration of various sensors and connection to online spectroscopic analysis.

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References

1. The Lancet (2020) Global health: time for radical change? Lancet 396:1129. https://doi.org/10.1016/S0140-6736(20)32131-0
2. Peli A, Pietra M, Giacometti F et al (2016) Survey on animal welfare in nine hundred and forty three Italian dairy farms. Ital J Food Saf 5:50–56. https://doi.org/10.4081/ijfs.2016.5832
3. Ho D, Quake SR, Mccabe ERB et al (2021) Enabling technologies for personalized and precision medicine. Trends Biotechnol 38:497–518. https://doi.org/10.1016/j.tibtech.2019.12.021.Enabling
4. Goetz LH, Schork NJ (2019) Personalized medicine: motivation, challenges and progress. Fertil Steril 109:952–963. https://doi.org/10.1016/j.fertnstert.2018.05.006.Personalized
5. Kieninger J, Weltin A, Flamm H, Urban GA (2018) Microsensor systems for cell metabolism-from 2D culture to organ-on-chip. Lab Chip 18:1274–1291. https://doi.org/10.1039/c7lc00942a
6. Jalili-Firoozinezhad S, Miranda CC, Cabral JMS (2021) Modeling the human body on microfluidic chips. Trends Biotechnol 39:838–852. https://doi.org/10.1016/j.tibtech.2021.01.004

7. Reardon S (2015) "Organs-on-chips" go mainstream. Nature 523:266. https://doi.org/10.1038/523266a
8. Lee SH, Jun BH (2019) Advances in dynamic microphysiological organ-on-a-chip: design principle and its biomedical application. J Ind Eng Chem 71:65–77. https://doi.org/10.1016/j.jiec.2018.11.041
9. Picollet-D'hahan N, Zuchowska A, Lemeunier I, Le Gac S (2021) Multiorgan-on-a-chip: a systemic approach to model and decipher inter-organ communication. Trends Biotechnol 39:788–810. https://doi.org/10.1016/j.tibtech.2020.11.014
10. Arjmand B, Goodarzi P, Mohamadi-Jahani F et al (2017) Personalized regenerative medicine. Acta Med Iran 55:144–149
11. Özdemir V, Arga KY, Aziz RK et al (2020) Digging deeper into precision/personalized medicine: cracking the sugar code, the third alphabet of life, and sociomateriality of the cell. OMICS 24:62–80. https://doi.org/10.1089/omi.2019.0220
12. Mirsadeghi S, Larijani B (2017) Personalized medicine: pharmacogenomics and drug development. Acta Med Iran 55:150–165
13. Carter MD, Simms GA, Weaver DF (2010) The development of new therapeutics for Alzheimer's disease. Clin Pharmacol Ther 88:475–486. https://doi.org/10.1038/clpt.2010.165
14. Lee VHL (2010) Personalized medicine: transforming drug development and healthcare. Ther Deliv 1:615–619. https://doi.org/10.4155/tde.10.68
15. Ando D, Meyer K (2017) Gene editing: regulatory and translation to clinic. Hematol Oncol Clin North Am 31:797–808. https://doi.org/10.1016/j.hoc.2017.06.002
16. Lee K, Conboy M, Park HM et al (2017) Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair. Nat Biomed Eng 1:889–901. https://doi.org/10.1038/s41551-017-0137-2
17. Lind JU, Busbee TA, Valentine AD et al (2017) Instrumented cardiac microphysiological devices via multimaterial three-dimensional printing. Nat Mater 16:303–308. https://doi.org/10.1038/nmat4782
18. Vegas AJ, Veiseh O, Doloff JC et al (2016) Combinatorial hydrogel library enables identification of materials that mitigate the foreign body response in primates. Nat Biotechnol 34:345–352. https://doi.org/10.1038/nbt.3462
19. Zimmer A, Tendler A, Katzir I et al (2017) Prediction of drug cocktail effects when the number of measurements is limited. PLoS Biol 15:1–16. https://doi.org/10.1371/journal.pbio.2002518
20. Chakradhar S (2017) Predictable response: finding optimal drugs and doses using artificial intelligence. Nat Med 23:1244–1247. https://doi.org/10.1038/nm1117-1244
21. Ma C et al (2021) Organ-on-a-chip: a new paradigm for drug development. Trends Pharmacol Sci 42(2):119–133. https://doi.org/10.1016/j.tips.2020.11.009
22. Haddrick M, Simpson PB (2019) Organ-on-a-chip technology: turning its potential for clinical benefit into reality. Drug Discov Today 24:1217–1223. https://doi.org/10.1016/j.drudis.2019.03.011
23. Theobald J, Ghanem A, Wallisch P et al (2018) Liver-kidney-on-chip to study toxicity of drug metabolites. ACS Biomater Sci Eng 4:78–89. https://doi.org/10.1021/acsbiomaterials.7b00417
24. Song JW, Daubriac J, Tse JM et al (2012) RhoA mediates flow-induced endothelial sprouting in a 3-D tissue analogue of angiogenesis. Lab Chip 12:5000–5006. https://doi.org/10.1039/c2lc40389g
25. Yang KS, Cheng YC, Jeng MS et al (2014) An experimental investigation of micro pulsating heat pipes. Micromachines 5:385–395. https://doi.org/10.3390/mi5020385
26. Wu Q, Liu J, Wang X et al (2020) Organ-on-a-chip: recent breakthroughs and future prospects. Biomed Eng Online 19:1–19. https://doi.org/10.1186/s12938-020-0752-0
27. Li YC, Lin MW, Yen MH et al (2015) Programmable laser-assisted surface microfabrication on a poly(vinyl alcohol)-coated glass chip with self-changing cell adhesivity for heterotypic cell patterning. ACS Appl Mater Interfaces 7:22322–22332. https://doi.org/10.1021/acsami.5b05978

28. Tibbe MP, Leferink AM, van den Berg A et al (2018) Microfluidic gel patterning method by use of a temporary membrane for organ-on-chip applications. Adv Mater Technol 3:1–6. https://doi.org/10.1002/admt.201700200
29. Mandenius CF (2018) Conceptual design of micro-bioreactors and organ-on-chips for studies of cell cultures. Bioengineering 5:56. https://doi.org/10.3390/bioengineering5030056
30. Young RE, Huh DD (2021) Organ-on-a-chip technology for the study of the female reproductive system. Adv Drug Deliv Rev 173:461–478. https://doi.org/10.1016/j.addr.2021.03.010
31. Raimondi I, Izzo L, Tunesi M et al (2020) Organ-on-a-chip in vitro models of the brain and the blood-brain barrier and their value to study the microbiota-gut-brain axis in neurodegeneration. Front Bioeng Biotechnol 7:435. https://doi.org/10.3389/fbioe.2019.00435
32. Miccoli B, Braeken D, Li Y-CE (2019) Brain-on-a-chip devices for drug screening and disease modeling applications. Curr Pharm Des 24:5419–5436. https://doi.org/10.2174/1381612825666190220161254
33. Mittal R, Woo FW, Castro CS et al (2019) Organ-on-chip models: implications in drug discovery and clinical applications. J Cell Physiol 234:8352–8380. https://doi.org/10.1002/jcp.27729
34. Park J, Lee BK, Jeong GS et al (2015) Three-dimensional brain-on-a-chip with an interstitial level of flow and its application as an in vitro model of Alzheimer's disease. Lab Chip 15:141–150. https://doi.org/10.1039/c4lc00962b
35. Wang Y, Wang L, Zhu Y, Qin J (2018) Human brain organoid-on-a-chip to model prenatal nicotine exposure. Lab Chip 18:851–860. https://doi.org/10.1039/c7lc01084b
36. Kilic O, Pamies D, Lavell E et al (2016) Brain-on-a-chip model enables analysis of human neuronal differentiation and chemotaxis. Lab Chip 16:4152–4162. https://doi.org/10.1039/c6lc00946h
37. Koo Y, Hawkins BT, Yun Y (2018) Three-dimensional (3D) tetra-culture brain on chip platform for organophosphate toxicity screening. Sci Rep 8:1–7. https://doi.org/10.1038/s41598-018-20876-2
38. Soscia D, Belle A, Fischer N et al (2017) Controlled placement of multiple CNS cell populations to create complex neuronal cultures. PLoS One 12:1–17. https://doi.org/10.1371/journal.pone.0188146
39. Huh D, Kim HJ, Fraser JP et al (2013) Microfabrication of human organs-on-chips. Nat Protoc 8:2135–2157. https://doi.org/10.1038/nprot.2013.137
40. Stucki AO, Stucki JD, Hall SRR et al (2015) A lung-on-a-chip array with an integrated bio-inspired respiration mechanism. Lab Chip 15:1302–1310. https://doi.org/10.1039/c4lc01252f
41. Blume C, Reale R, Held M et al (2015) Temporal monitoring of differentiated human airway epithelial cells using microfluidics. PLoS One 10:1–13. https://doi.org/10.1371/journal.pone.0139872
42. Humayun M, Chow CW, Young EWK (2018) Microfluidic lung airway-on-a-chip with arrayable suspended gels for studying epithelial and smooth muscle cell interactions. Lab Chip 18:1298–1309. https://doi.org/10.1039/c7lc01357d
43. Yang X, Li K, Zhang X et al (2018) Nanofiber membrane supported lung-on-a-chip microdevice for anti-cancer drug testing. Lab Chip 18:486–495. https://doi.org/10.1039/c7lc01224a
44. Benam KH, Villenave R, Lucchesi C et al (2016) Small airway-on-a-chip enables analysis of human lung inflammation and drug responses in vitro. Nat Methods 13:151–157. https://doi.org/10.1038/nmeth.3697
45. Tan Q, Choi KM, Sicard D, Tschumperlin DJ (2017) Human airway organoid engineering as a step toward lung regeneration and disease modeling. Biomaterials 113:118–132. 10.1016/j.biomaterials.2016.10.046
46. Peng J, Rochow N, Dabaghi M et al (2018) Postnatal dilatation of umbilical cord vessels and its impact on wall integrity: prerequisite for the artificial placenta. Int J Artif Organs 41:393–399. https://doi.org/10.1177/0391398818763663

47. Dabaghi M, Fusch G, Saraei N et al (2018) An artificial placenta type microfluidic blood oxygenator with double-sided gas transfer microchannels and its integration as a neonatal lung assist device. Biomicrofluidics 12:044101. https://doi.org/10.1063/1.5034791
48. Xu Z, Gao Y, Hao Y et al (2013) Application of a microfluidic chip-based 3D co-culture to test drug sensitivity for individualized treatment of lung cancer. Biomaterials 34:4109–4117. https://doi.org/10.1016/j.biomaterials.2013.02.045
49. Ronaldson-Bouchard K, Vunjak-Novakovic G (2018) Organs-on-a-chip: a fast track for engineered human tissues in drug development. Cell Stem Cell 22(3):310–324. https://doi.org/10.1016/j.stem.2018.02.011
50. Mittal R et al (2018) Organ-on-chip models: implications in drug discovery and clinical applications. J Cell Physiol. https://doi.org/10.1002/jcp.27729
51. Skardal A, Murphy SV, Devarasetty M et al (2017) Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. Sci Rep 7:1–16. https://doi.org/10.1038/s41598-017-08879-x
52. An F, Qu Y, Liu X et al (2015) Organ-on-a-chip: new platform for biological analysis. Anal Chem Insights 10:39–45. https://doi.org/10.4137/Aci.s28905
53. Bronte V, Pittet MJ (2013) The spleen in local and systemic regulation of immunity. Immunity 39:806–818. https://doi.org/10.1016/j.immuni.2013.10.010
54. Rigat-Brugarolas LG, Elizalde-Torrent A, Bernabeu M et al (2014) A functional microengineered model of the human splenon-on-a-chip. Lab Chip 14:1715–1724. https://doi.org/10.1039/c3lc51449h
55. Kang JH, Super M, Yung CW et al (2014) An extracorporeal blood-cleansing device for sepsis therapy. Nat Med 20:1211–1216. https://doi.org/10.1038/nm.3640
56. Shanti A, Teo J, Stefanini C (2018) In vitro immune organs-on-chip for drug development: a review. Pharmaceutics 10:278. https://doi.org/10.3390/pharmaceutics10040278
57. Lee SH, Sung JH (2018) Organ-on-a-chip technology for reproducing multiorgan physiology. Adv Healthc Mater 7:1–17. https://doi.org/10.1002/adhm.201700419
58. Boeri L, Izzo L, Sardelli L et al (2019) Advanced organ-on-a-chip devices to investigate liver multi-organ communication: focus on gut, microbiota and brain. Bioengineering 6:91. https://doi.org/10.3390/bioengineering6040091
59. Wang L, Wu J, Chen J et al (2021) Advances in reconstructing intestinal functionalities in vitro: from two/three dimensional-cell culture platforms to human intestine-on-a-chip. Talanta 226:122097. https://doi.org/10.1016/j.talanta.2021.122097
60. Lucchetti M, Kaminska M, Oluwasegun AK et al (2021) Emulating the gut–liver axis: dissecting the microbiome's effect on drug metabolism using multiorgan-on-chip models. Curr Opin Endocr Metab Res 18:94–101. https://doi.org/10.1016/j.coemr.2021.03.003
61. Korea S (2020) A body-on-a-chip (BOC) system for studying gut-liver interaction. Methods Cell Biol 158:1–10
62. Fan H, Demirci U, Chen P (2019) Emerging organoid models: leaping forward in cancer research. J Hematol Oncol 12:1–10. https://doi.org/10.1186/s13045-019-0832-4
63. Jang KJ, Mehr AP, Hamilton GA et al (2013) Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment. Integr Biol (United Kingdom) 5:1119–1129. https://doi.org/10.1039/c3ib40049b
64. Musah S, Dimitrakakis N, Camacho DM et al (2018) Directed differentiation of human induced pluripotent stem cells into mature kidney podocytes and establishment of a Glomerulus Chip. Nat Protoc 13:1662–1685. https://doi.org/10.1038/s41596-018-0007-8
65. Eddie SL, Kim JJ, Woodruff TK, Burdette JE (2014) Microphysiological modeling of the reproductive tract: a fertile endeavor. Exp Biol Med 239:1192–1202. https://doi.org/10.1177/1535370214529387
66. Xiao S, Coppeta JR, Rogers HB et al (2017) A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle. Nat Commun 8:1–13. https://doi.org/10.1038/ncomms14584

67. Li WX, Liang GT, Yan W et al (2013) Artificial uterus on a microfluidic chip. Chinese J Anal Chem 41:467–472. https://doi.org/10.1016/S1872-2040(13)60639-8
68. Laronda MM, Burdette JE, Kim JJ, Woodruff TK (2013) Recreating the female reproductive tract in vitro using iPSC technology in a linked microfluidics environment. Stem Cell Res Ther 4:1–5. https://doi.org/10.1186/scrt374
69. Gnecco JS, Pensabene V, Li DJ et al (2017) Compartmentalized culture of perivascular stroma and endothelial cells in a microfluidic model of the human endometrium. Ann Biomed Eng 45: 1758–1769. https://doi.org/10.1007/s10439-017-1797-5
70. Mancini V, Pensabene V (2019) Organs-on-chip models of the female reproductive system. Bioengineering 6:1–16. https://doi.org/10.3390/bioengineering6040103
71. Blundell C, Tess ER, Schanzer ASR et al (2016) A microphysiological model of the human placental barrier. Lab Chip 16:3065–3073. https://doi.org/10.1039/c6lc00259e
72. Rothbauer M, Bachmann BEM, Eilenberger C et al (2021) A decade of organs-on-a-chip emulating human physiology at the microscale: a critical status report on progress in toxicology and pharmacology. Micromachines 12:470. https://doi.org/10.3390/mi12050470
73. Sutterby E, Thurgood P, Baratchi S et al (2020) Microfluidic skin-on-a-chip models: toward biomimetic artificial skin. Small 16:1–17. https://doi.org/10.1002/smll.202002515
74. Alexander FA, Eggert S, Wiest J (2018) Skin-on-a-chip: transepithelial electrical resistance and extracellular acidification measurements through an automated air-liquid interface. Genes (Basel) 9:114. https://doi.org/10.3390/genes9020114
75. Sriram G, Alberti M, Dancik Y et al (2018) Full-thickness human skin-on-chip with enhanced epidermal morphogenesis and barrier function. Mater Today 21:326–340. https://doi.org/10.1016/j.mattod.2017.11.002
76. Mori N, Morimoto Y, Takeuchi S (2017) Skin integrated with perfusable vascular channels on a chip. Biomaterials 116:48–56. https://doi.org/10.1016/j.biomaterials.2016.11.031
77. Alberti M, Dancik Y, Sriram G et al (2017) Multi-chamber microfluidic platform for high-precision skin permeation testing. Lab Chip 17:1625–1634. https://doi.org/10.1039/c6lc01574c
78. Wufuer M, Lee GH, Hur W et al (2016) Skin-on-a-chip model simulating inflammation, edema and drug-based treatment. Sci Rep 6:1–12. https://doi.org/10.1038/srep37471
79. Lukács B, Bajza Á, Kocsis D et al (2019) Skin-on-a-chip device for ex vivo monitoring of transdermal delivery of drugs—design, fabrication, and testing. Pharmaceutics 11:445. https://doi.org/10.3390/pharmaceutics11090445
80. Van Der Meer AD, Van Den Berg A (2012) Organs-on-chips: breaking the in vitro impasse. Integr Biol 4:461–470. https://doi.org/10.1039/c2ib00176d
81. Pires De Mello CP, Carmona-Moran C, McAleer CW et al (2020) Microphysiological heart-liver body-on-a-chip system with a skin mimic for evaluating topical drug delivery. Lab Chip 20:749–759. https://doi.org/10.1039/c9lc00861f
82. Satoh T, Sugiura S, Shin K et al (2018) A multi-throughput multi-organ-on-a-chip system on a plate formatted pneumatic pressure-driven medium circulation platform. Lab Chip 18:115–125. https://doi.org/10.1039/c7lc00952f
83. Miller PG, Shuler ML (2016) Design and demonstration of a pumpless 14 compartment microphysiological system. Biotechnol Bioeng 113:2213–2227. https://doi.org/10.1002/bit.25989
84. Oleaga C, Riu A, Rothemund S et al (2018) Investigation of the effect of hepatic metabolism on off-target cardiotoxicity in a multi-organ human-on-a-chip system. Biomaterials 182:176–190. https://doi.org/10.1016/j.biomaterials.2018.07.062
85. Materne EM, Ramme AP, Terrasso AP et al (2015) A multi-organ chip co-culture of neurospheres and liver equivalents for long-term substance testing. J Biotechnol 205:36–46. https://doi.org/10.1016/j.jbiotec.2015.02.002
86. Ong LJY, Ching T, Chong LH et al (2019) Self-aligning Tetris-Like (TILE) modular microfluidic platform for mimicking multi-organ interactions. Lab Chip 19:2178–2191. https://doi.org/10.1039/c9lc00160c

87. Kimura H, Ikeda T, Nakayama H et al (2015) An on-chip small intestine–liver model for pharmacokinetic studies. J Lab Autom 20:265–273. https://doi.org/10.1177/2211068214557812
88. Van Den Berg A, Mummery CL, Passier R, Van der Meer AD (2019) Personalised organs-on-chips: functional testing for precision medicine. Lab Chip 19:198–205. https://doi.org/10.1039/c8lc00827b
89. Tsai M, Kita A, Leach J et al (2012) In vitro modeling of the microvascular occlusion and thrombosis that occur in hematologic diseases using microfluidic technology. J Clin Invest 122:408–418. https://doi.org/10.1172/JCI58753
90. Pandian NKR, Mannino RG, Lam WA, Jain A (2018) Thrombosis-on-a-chip: prospective impact of microphysiological models of vascular thrombosis. Curr Opin Biomed Eng 5:29–34. https://doi.org/10.1016/j.cobme.2017.12.001
91. Orlova V, Mummery C (2016) SnapShot: key advances in hiPSC disease modeling. Cell Stem Cell 18:422. https://doi.org/10.1016/j.stem.2016.02.013

Development and Application of Microfluidics in Organoid Formation

14

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Abstract

Understanding human physiology and thereby finding interventions during pathological conditions has been the path of research for most of the researchers if not all during the recent decades. The studies have been successful with the help of animal models and cell culture models, conventionally. Though these numerous scientific advances have given greater insights, their applications in modelling human physiology and pathology are limited. Owing to either the existing physiological differences between humans and animals or the simplicity of 2D (two-dimensional) cell culture, these conventional methods fail to provide the complexity and cellular diversities of tissues. These limitations and the need for detailed studies using the near-perfect organ/tissue/cellular level studies have given scope for the development of 3D cell culture techniques with the recent advancements in materials and manufacturing techniques like bioprinting combined with cell technology to generate 3D cell cultures as organoid models. Microfluidics has provided unprecedented opportunities in constructing cellular engineering and cell microenvironments and has slowly been replacing the conventional cell cultures and cellular assays. The flexibility in the engineering of the microfluidic device has provided opportunities to tailor specific needs required for individual cell types or cell cocultures. These unique features, tools and techniques have been represented in this chapter. The way forward with 3D

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organoid models has also been highlighted as it is becoming the next big thing in the field of health science research.

Keywords

Microfluidics · Organoid cultures · 3D cell culture · Stem cell culture

14.1 Introduction

Human physiology and pathology have long been understood with help of animal models and conventional two-dimensional (2D) cell culture models [1]. Though these models have promoted and aided numerous scientific advances, their applications in modelling human physiology and pathology are limited [2]. If animal models are limited inherently in mimicking human-specific biology due to existing physiological differences between humans and animals, the simplicity of 2D cell culture fails to reflect the complexity and cellular diversities of tissues in vivo. In addition, owing to ethical considerations, our access to adult or human embryonic tissues has been minimal [3]. These limitations have created a demand and as well paved the way for advancements in materials and manufacturing techniques combined with cell technology to generate 3D (three-dimensional) cell cultures [4].

Organoids are three-dimensional constructs composed of multiple cell types originating from stem cells by means of self-organization. Organoids are capable of simulating the architecture and functionality of native organs [5]. They can be generated with the use of somatic cells, adult stem cells, or pluripotent stem cells [6]. The advantages of organoids over the traditional 2D cultures and animal models are as follows:

- Their ability to display near-physiological cellular composition and behaviours.
- They are more suitable for biobanking and high-throughput screens due to their ability to maintain genome stability even after undergoing extensive expansion in culture.
- They can reduce the experimental complexity.
- They are amenable to real-time imaging techniques.
- More importantly, they enable the study of aspects of human development and disease that are not easily or accurately modelled in animals (Fig. 14.1).

14.2 Microfluidic Technology Based Organoid Models

Microfluidic approaches have advantages over other in vitro culture models because of fine control over physical and chemical parameters, the design of complex tissue constructs and the use of multiple biomaterials to better mimic the in vivo organs [7] (Fig. 14.2). Two areas with the potential to transform organoid production are micropatterning and microfluidics [8]. Microfabricated and microfluidic devices

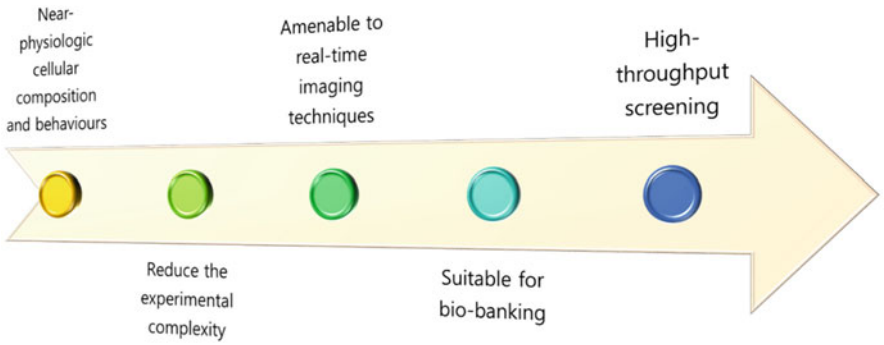

Fig. 14.1 Advantages of organoids over traditional 2D cultures and animal models

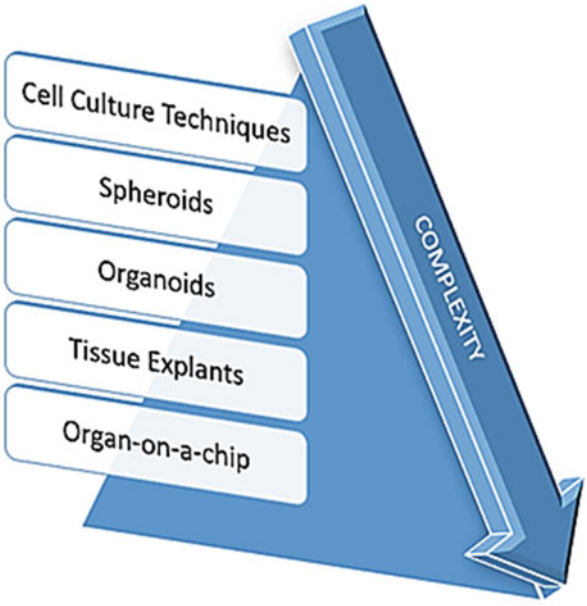

Fig. 14.2 Achieving the complexity in models by the application of microfluidics to produce organoids

have allowed for the arrayed production of size-controlled culture areas that yield more uniform organoids and spheroids for a higher throughput at a minimal cost in addition to an efficient supply of nutrients and growth factors. These miniature-sized devices are also called organ-on-a-chip device [9].

Microfluidic systems have facilitated the generation of culture systems that mimic early human organ development at a scale that is not achievable with conventional cell culture methods [10] (Fig. 14.3). One such study focused on assessing the performance of a microfluidic chip where induced human pluripotent stem cells (ihPSCs) developed into organoids with intermediates such as embryoid bodies and

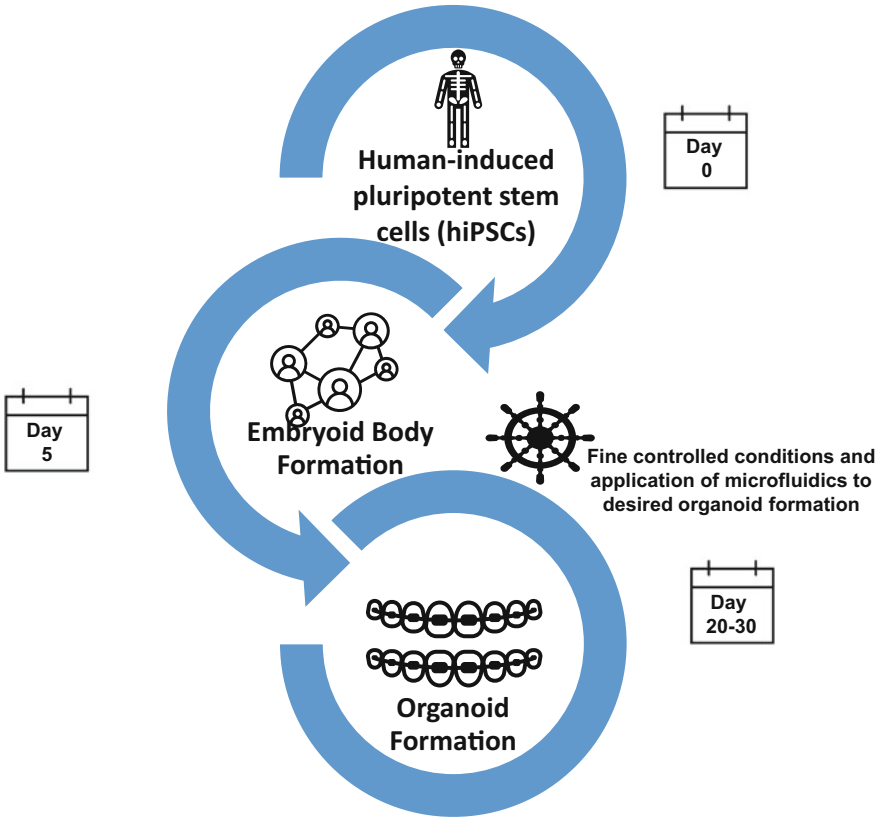

Fig. 14.3 Processing of human-induced pluripotent stem cells (hiPSCs)

neuroectoderm [11]. Also, embryoid bodies were mixed with Matrigel to allow the exchange of gases in addition to cortical differentiation [12].

Matrigel-loaded intestinal organoid culture systems are not physiologically compatible because most of the toxic degraded products accumulate in the central lumen due to lack of functional peristalsis in vitro [13]. Microfluidic intestinal culture systems are promising due to their ability to form villi-like structures. This organ of a chip contains an epithelial and vascular channel separated by a 50 μm porous PDMS membrane [14]. In addition to this, vacuum chambers on either side of the channel create suction, as a result of which the microchannels stretch and relax, which facilitates peristalsis, similar to an in vivo intestinal tissue [15].

Another important aspect of any organoid is the ability to undergo vascularization. Successful vascularization of human lung fibroblast spheroids of a diameter of 600 μm grown in a microfluidic chip has been performed [16]. The study has used a microchip device such that the middle channel consisted of the lung spheroids while the adjacent side channels were seeded with human umbilical vein endothelial cells

(HUVECs) [17]. This has allowed cell migration and proliferation, thereby allowing HUVECs to form a network around and within the lung spheroids.

In an attempt to engineer perineural vascular plexus to study developmental toxicity, a well-fabricated brain organoid containing a dense network of human embryonic stem cell (hESC)-derived endothelial cells (ECs), neural progenitor cells and microglia (MG) with primary pericytes (PCs) and an overlying neural layer in synthetic hydrogels embedded in a microfluidic device has been developed. Such a culture has shown prominent functional properties, such as increased neurotrophic factor secretion and differential metabolite secretion [18]. Through the development and utilization of these microfluidic systems, in vitro disease modelling, regenerative therapy, and drug screening can be done, which shows that these microfluidic systems have potential biomedical, pharmaceutical and toxicological applications.

14.3 Tools and Techniques for Organoid Cultures

For organoid development, generally a single induced or embryonic pluripotent stem cell (iPSC or ESC) or progenitor cells are isolated from adult tissues, which upon cell expansion undergo self-organization into a homogeneous cell system. Self-organization is the ability to organize and mimic neighbouring cells to form a tissue construct upon environmental stimulus by the process of differentiation. In addition, paracrine interactions facilitate organ-specific architectures [19]. Some methods that have helped lay the foundation of organoid culturing are discussed in this section.

14.3.1 ECM Scaffold Method

Most organoids are produced by the simple expansion of stem cells in 3D structures of extracellular matrix (ECM)-derived protein mixtures (from Engelbreth–Holm–Swarm mouse sarcoma), also called a Matrigel [8]. The desired cell type is obtained by antibody staining followed by fluorescence-activated cell sorting (FACS), which can later be plated on our protein mixture. Cell–ECM interactions promote tissue differentiation and grouping, as a result of which a tissue-specific lineage is derived that generates phenotypically and genetically similar organoids [20]. Not just Matrigel, but also other ECM agents such as alginate, collagen, laminin, fibrin and polyethylene glycol (PEG) can be cross-linked with the cell suspension of our choice [21]. Although synthetic hydrogels are much more optimized and well characterized in terms of mechanical and biochemical properties, the need for natural hydrogels is much more evident because of their ability to mimic in vivo ECM scaffolds. Interestingly, a study in 2019 focused on developing a GMP-compatible ECM self-gelling hydrogel from decellularized porcine small intestine mucosa/submucosal tissues that are as mechanically stable as their synthetic commercially available counterparts and respond well to signals of cells derived from other endoderm-derived tissues such as liver, stomach and pancreas [22].

14.3.2 Spinning Bioreactor Method

Here, cells are generally placed in a container that is constantly stirred using paddles driven by a single electric motor or a spinning bar [23]. Spin omega is one such model bioreactor that is developed by Lancester et al. for culturing brain-region-specific organoids. It is not only versatile and efficient at giving an output but also has added advantages like lesser media consumption (3 mL of media per well compared to 75–100 mL of media per spinner) [24]. Another general advantage of spinner flasks is the inbuilt agitation that prevents cellular aggregation and cell adherence to petri dish surfaces. Improvisations to the existing technology include durable motors (DC 12 V 100 RPM gear motor) to withstand harsh temperature ranges (up to 70 °C) and humidity (maximum 90%), autoclavable stainless steel screws, nuts and washers in order to reduce the oxidation levels of the metal [25].

In one study, neuroectodermal tissues embedded in Matrigel droplets were also formed using a spinning bioreactor. Here, human pluripotent stem cells grown in a low fibroblast growth factor (FGF) signalling media have been induced to form small embryonic bodies that have further been induced to form neuroepithelial tissues with close resemblance to the in vivo cortex. Such induced brain tissues are also called cerebral organoids. In fact, these tissues have expressed markers of different cortical layers that have a high level of similarity to the in vivo human brain in the early stages of development [24].

On similar lines, a cost-effective robust method has been developed to produce kidney organoids from induced pluripotent stem cells (iPSCs) that involves the formation of embryoid bodies in the presence of CHIR99021 (CHIR) and Knockout serum replacement (KSOR) as a supplement. This method, involving spinner flasks, resulted in the formation of organoids containing prospective proximal and distal convoluted tubule segments, endothelial cells and interstitial cells, which upon comparison with foetal human kidneys suggests that 14-day-old organoid tissue closely resembles late capillary loop stage nephrons [26].

14.3.3 Magnetic Levitation

Magnetic levitation (ML) involves incubating target cells with magnetic nanoparticles to induce a magnetic component to the cells [27]. A magnetic lid that is placed on the culture plate, attracted the nanoparticles that were ingested by cells, to form a liquid-air suspension. They then aggregate to form a larger 3D network that proliferates to give rise to organoids. Another modification to this includes coating gold or iron oxide nanoparticles with hydrogels overnight [28].

Saliva secreting organoids have been developed using magnetic 3D levitation (M3DL) to treat salivary gland (SG) hypofunction and oral dryness that is induced by radiotherapy for head and neck cancers. A culture system like this levitates magnetized primary SG-derived cells (SGDCs), allowing them to produce their own extracellular matrix proteins and SG-like organoids in epithelial differentiation conditions in a week's time. Such well-differentiated, uniform spheres can prevent

SG dysfunction that affects assimilation processes such as chewing and swallowing [29]. In another interesting case, the mobility of mesenchymal stem cells (MSCs) as cellular vectors in response to injury or regenerative response has been studied. MSCs have been incorporated with fluorescently labelled magnetic nanoparticles that upon levitation have proliferated to organoids. Here, the movement of individual MSCs has been tracked in response to interleukin-6, which stimulates the recruitment of MSCs that stimulate tissue repair at the site of the wound [30].

14.3.4 Bioprinting

This method involves the layer-by-layer fabrication of biomaterials/scaffolds and living cells in a precise 3D architecture using computer-aided transfer and build-up processes. Bioinks of desired characteristics such as precise viscosity, efficient cross-linking and appropriate surface tension as well as bioprinters of an appropriate resolution and high speed are important components of any bioprinting procedure [19]. An amalgamation of alginate and gelatine is generally used for extrusion-based bioprinting to combine the thermo-sensitive properties of gelatine with the chemical cross-linking capabilities of alginate. Cross-linking of the bioinks during or immediately after the bioprinting promotes stabilization of the printed tissue construct. Natural bioinks have lesser mechanical strength, whereas synthetic biopolymer-based bioinks have poor compatibility and toxic end products upon degradation. Therefore, studies have tried to incorporate a hybrid of both natural and synthetic derivatives to facilitate 3D bioprinting [19]. A good example of this is a hybrid bioink system consisting of polycaprolactone and alginate with few growth factors like TGF-Beta, that has been used for cartilage regeneration both in vitro and in vivo [31].

The kind of bioprinter used to fabricate organoids also contributes to the overall construct. Generally, thermal and piezoelectric and thermal-based inkjet bioprinters and laser bioprinters are used to assemble tissues because of higher cell viability and resolution. Even pressure-driven extrusion methods that print with higher cell densities have been used [32] to print human iPSCs cocultured with irradiated chondrocytes in a bioink composed of nanofibrillated cellulose with alginate. The bioink here maintained pluripotency and cartilaginous tissue for over 5 weeks. The microelectromechanical system (MEMS) has created a lucrative platform to develop microfabricated organoids allowing higher output and low cost in mass production [33].

14.3.5 3D Cell Culture Techniques and 3D Organoids, Stem Cell 3D Organoids

14.3.5.1 3D Cell Culture Techniques

The inadequacies of traditional two-dimensional (2D) cell culture models are overcome by 3D cell culture techniques, which are substantially more informative than

2D systems. They readily replace or minimize the amount of in vivo investigations, which are associated with a slew of ethical issues in the development of new treatments [34]. Two-dimensional cell cultures are performed on rigid plastic or glass surfaces, and they do not provide a physiologically realistic environment, resulting in alterations in cell function. Extracellular matrix (ECM) is not present in cells grown in 2D culture. They also differ from in vivo cell development. They do not respond to biochemical signals because they cannot migrate, which also results in variation in drug sensitivity and toxicity [35]. Top-down and bottom-up techniques are utilized in 3D systems in general. In a top-down approach, the desired cells are cultured in a pre-prepared tailor made scaffolds of specific size and shape. Single cells or spheroids are employed as building blocks to construct complex tissue architectures in the 'bottom-up' approach. Although specific techniques differ, a combination of them is utilized to improve the morphological, functional and microenvironmental features of bioengineered human tissues and organs [36]. Depending on the sort of experiment, 3D culture offers a variety of cell culture methodologies. Hydrogel-based support, polymeric hard material-based support, hydrophilic glass fibre and organoids are examples of scaffold-based approaches that offer a variety of benefits. Hydrogels have the capacity to resemble the ECM. They allow soluble molecules such as cytokines and growth factors to pass through the tissue-like gel [1]. Different kinds of 3D stem culture techniques are discussed next.

14.3.5.2 Liquid Overlay

Liquid overlay cultures are stationary and create a single spheroid at the centre of each well, and phase-contrast light microscopy can be used to easily track their growth. Using sophisticated equipment such as the Celigo cytometer, images of the spheroids in each well may be taken and analysed [37]. The extracellular matrix that holds spheroids together is naturally secreted by the cells when they are formed using the liquid overlay approach [38]. This method is the best approach for growing reproducible 3D cell cultures with a uniform, well-defined shape that can be used for automated high-throughput screens and data mining [35]. Proteins and ECM elements, such as collagen, fibrin, hyaluronic acid, or Matrigel, are used to make naturally derived hydrogels for cell culture, as well as materials generated from other biological sources, such as chitosan, alginate, gelatin, agarose, or dextran [39]. It is inexpensive, simple to handle, and does not necessitate the use of specialized equipment. Shear stresses are kept to a minimum. Liquid overlay technique helps in cell culturing over a long period of time. This faciliates large spheroid manufacturing [40].

14.3.5.3 Hanging-Drop Method

The typical hanging-drop approach involves placing cells suspended in a culture medium on the underside of petri dish covers (Fig. 14.4). The cells gather at the drop's tip, aggregate spontaneously, and form spheroids [41]. Mesenchymal stem cells (MSCs) rapidly collect in hanging-drop cultures, merging into a single central sphere at the drop's apex. MSCs grown in hanging-drop cultures have been utilized

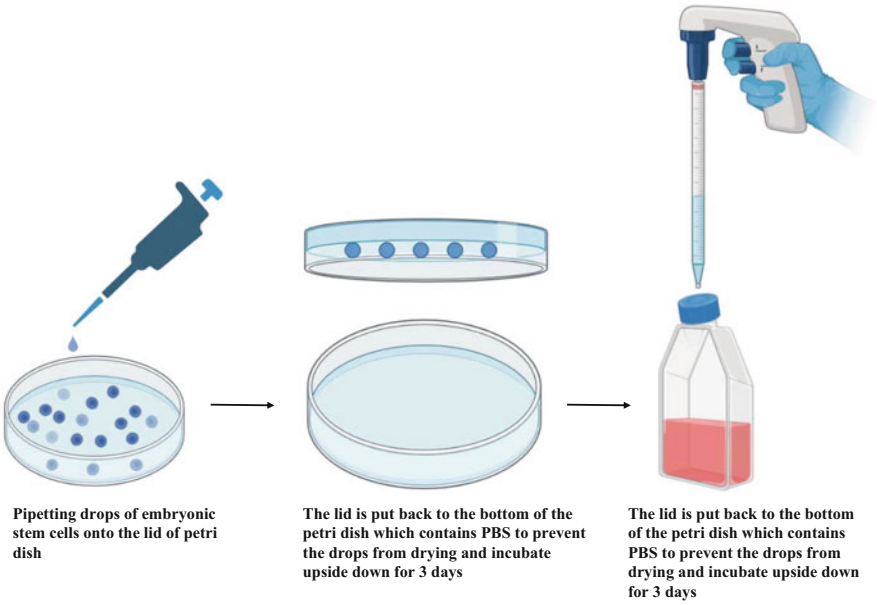

Fig. 14.4 Hanging-drop approach of 3D cell culture technique

to make spheres, with certain advantages over conventional sphere generation techniques [42]. The size of the sphere can be readily changed by adjusting the drop size or cell concentration. For experimental and small animal model-scale work, no specialized or expensive equipment is required, as large amounts of spheres can be easily formed using multichannel pipettors and harvested by simply scraping the culture dish lid and collecting the spheres with a pipette for centrifugation [43].

14.3.5.4 Bioreactor

A bioreactor is an instrument that enables the reliable and cost-effective generation of cells in a controlled environment. A bioreactor for the generation of 3D spheroid can be divided into five kinds, that is, mechanical force systems, spinner flask bioreactors, rotational culture systems, perfusion bioreactors and spinner flask bioreactors [44]. The main premise of bioreactor-based 3D culture systems is that a cell suspension with the optimal cell density is continuously agitated into the chamber, by gently stirring, rotating the chamber, or pumping culture medium via a scaffold. To provide nutritional circulation, metabolic waste ejection and uniformity of the physical and chemical components within the bioreactors, bioreactors are supplied with medium flowing systems. As a result, bioreactor-based cell culture models are suited for large-scale biomolecule production, such as antibodies or growth factors, as well as intensive cell proliferation.

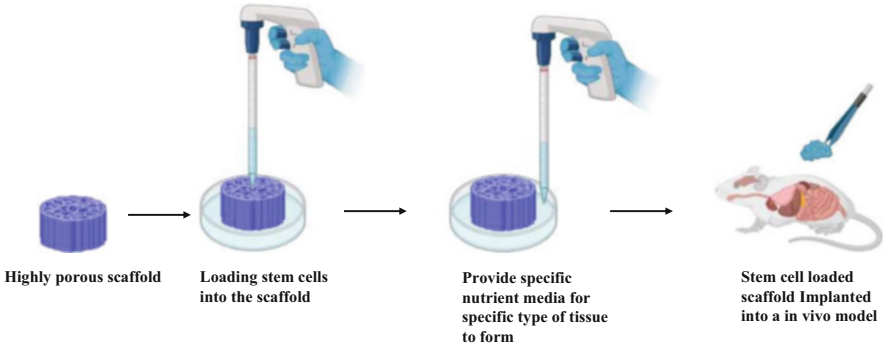

Fig. 14.5 Culturing stem cells in 3D biomaterial scaffolds for implantation in in vivo models

14.3.5.5 Scaffolds

Culturing stem cells in 3D biomaterial scaffolds allows researchers to properly imitate the microenvironments, giving them an advantage over traditional 2D polystyrene culture methods and a possibility to engineer replacement tissues [45] (Fig. 14.5). By enabling more accurate creation of cell polarity in the environment and exhibiting biochemical and mechanical qualities identical to soft tissue, 3D biomaterial scaffolds can more closely imitate the microenvironments present in vivo [46]. In the context of tissue engineering, scaffolds with well-defined, customizable structural characteristics and mechanical properties are critical for increasing stem cell therapeutic and regenerative efficacy. The development of 3D scaffolds for tissue engineering has taken a lot of time and effort [36]. 3D scaffolds for tissue engineering have taken a lot of time and effort to design. Electrospinning, lithography, microfabrication and self-assembly techniques, for example, have all been extensively investigated for fabricating 3D scaffolds suitable for specific tissue applications. Porous scaffolds used for culturing are basically made from a variety of biopolymers, including collagen [47], gelatin [48, 49], silk [50], chitosan [51] and alginate [52].

14.3.5.6 3D Organoids

Organoids are three-dimensional (3D) cell constructs generated in vitro from stem cells, primarily extracted from biopsies or pluripotent stem cells, that mimic important aspects of the native organ in development and performance [5]. Organoids' ability to produce sophisticated 3D structures that resemble organs is transforming developmental and stem cell biology [2]. Despite their widespread use in basic research, organoids are now used only in drug testing in translational biomedicine [53]. Organoids have facilitated existing animal model systems, fundamental biological/medical research and drug development into a more appropriate human body mimicking setting. These applications have been growing rapidly recently. However, compared to established cell lines and animal models, organoid technology is taking its initial steps to be implemented worldwide, with many obstacles to

overcome [2]. The efficacy of animal models in human disease research is limited by differences in microbiota and pathogen composition between animal models and humans, as well as the failure of certain phenomena found in mice to translate directly to humans. A human organoid culture can be created in a few weeks or months with a high success rate, supporting the use of patient-derived organoids in personalized medicine to give comprehensive tailored data, including unique mutation profiles and treatment responses [54].

14.3.5.7 Stem Cell 3D Organoids

Organoid systems, as opposed to tissue explants, can imitate similar cell–cell and cell–matrix interactions while retaining the ability to cultivate for long periods due to the presence of essential survival signalling cues. Organoids have been created from both pluripotent stem cells (PSCs) and adult stem cells (ASCs) using biochemical and physical cues to imitate tissue growth and homeostasis [55]. Organoids have niche components that are either produced by the cells, such as autocrine, paracrine and juxtacrine signals, or are given to the system exogenously, such as ECM substrates, small molecules and growth factors [56].

Organoids have their own set of constraints. Even though several organoid preparations comprise a variety of cell types, research into how effectively these models mirror their in vivo counterparts is still underway. Many obvious components, such as the vasculature and immune cells, are missing, and so the model cannot be used to examine processes or diseases that require them. The organoid may not always match the original organ model perfectly. The cells of the human pluripotent stem cell (hPSC)-derived organoids are young, and their expression profiles closely resemble those of foetal tissue in most instances [57]. Cancer models at the preclinical stage are necessary. One of the key causes for this is inter- and intra-patient tumour heterogeneity, which is important to understand cancer biology, especially tumour heterogeneity. Animal models cannot entirely reflect the genetic characteristics of human cancers and are unable to adequately capture tumour heterogeneity, making them unsuitable for understanding the oncogenic process. Organoids can be created from patient biopsies and resections to replicate the original cancer tissue. Tumour organoids (tumoroids) are easily produced from tumour tissue and propagated under controlled conditions [58]. Organoid biobanking, disease modelling, medication toxicity testing, tailored therapy, host–microbe interaction investigations, and omics analysis of healthy and diseased organoids are just a few of the clinical applications for stem cell-derived organoids. Furthermore, organoids produced from disease tissue can be treated with targeted gene therapy using the CRISPR/Cas9 system [59]. They have reaffirmed the idea of ‘self-organization’ in which pluripotent cells can generate complex neural tissues similar to forebrain-derived cortical neuronal networks of their in vivo counterparts without any external inputs by using a serum-free culture medium [60].

14.4 Conclusion

For disease modelling, human stem cell-derived organoids provide an attractive platform because 3D bioprinted organoids that reproduce both cell-to-cell and cell–matrix interactions can address the limitations of cell lines and animal models, such as the inability to study cell migration and proliferation studies as well as limited efficiency of 2D tissues to mimic biologically relevant organs. The organoids can be treated with various inflammatory stimuli for disease modelling and mechanistic studies. In fact, human gastric organoids have been developed to study *H. pylori* infections. The relative stability of these organoids has been assessed using expression markers as well as epithelial responses to microarray and PCR analyses [61].

One of the biggest limitations of organoid cultures to generate fully functional tissues is that organoids cease to proliferate and develop a necrotic core upon reaching a certain size. Hence, along with bio-fabrication, achieving adequate vascularization and perfusion of the bioprinted organoid through the incorporation of endothelial cells along with the stem cells/organoids or by culturing the organoids onto 3D printed vascular structures or by using microfluidic devices is essential. The use of endothelial cells to attain vascularization and angiogenesis of human pluripotent stem cell-derived organoids ensures oxygen and nutrient distribution in large organoids, thus contributing to the maturation of adult-like organoids through paracrine interactions [19].

Apparently, the reproducibility of organoids remains challenging because of a complex multistep procedure that depends on multiple variables such as cell type, cellular state and growth [62]. A major limitation of the ECM scaffold method discussed above is the ability to maintain a consistent generation of scaffolds that represent the composition of the ECM that is naturally present in the original tissue [21]. One of the reasons could be reduced organoid growth after several passages, which was actually an issue faced by Giobbe et al. [22], the methodology of which is discussed above. It was likely due to the accumulation of stiff ECM leftovers from previous passages. Many organoid models, although have the ability to mimic in vivo tissues, lack the ability to tolerate shear stress by blood flow [20] or do not take an account of the absence of microglia, the in-house macrophages of the brain, because of which a neuro-inflammatory component to these brain organoids is lacking. Since microglial cells are important for the pathophysiology of many neurological disorders like Alzheimer's, it is necessary to consider a microglial component to brain organoids in the future [63, 64]. Even with respect to kidney organoids, many standardized protocols have limitations because of the high cost of culture reagents and supplements such as fibroblast growth factor 9 (FGF9) and B27 (a serum-free supplement), thus hindering the large-scale production of kidney organoids. A major limitation of magnetically levitated organoids, the principle of which is discussed above, can be due to nanoparticles that are used to induce magnetism because nanoparticles can be costly and tend to induce toxic effects on cells if used in large quantities [65]. Although there are a number of drawbacks that

are hindering the standardization of organoids, it remains one of the most promising approaches to study biotherapeutics, disease modelling and drug screening mainly because of its ability to mimic in vivo human organs in in vitro conditions and its potential to replace the extensive use of animals as models. Modern research focuses on developing novel biomaterials that act as permeable synthetic frameworks intended to permit the perfusion of liquids and gases and promote cellular communication and cell viability with minimum inflammation and toxicity. Such scaffolds can take microfluidic organoid culture systems to another level [46].

References

1. Jensen C, Teng Y (2020) Is it time to start transitioning from 2D to 3D cell culture? Front Mol Biosci 7:33
2. Kim J, Koo B-K, Knoblich JA (2020) Human organoids: model systems for human biology and medicine. Nat Rev Mol Cell Biol 21(10):571–584
3. Volarevic V, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N et al (2018) Ethical and safety issues of stem cell-based therapy. Int J Med Sci 15(1):36–45
4. Ravi M, Paramesh V, Kaviya SR, Anuradha E, Solomon FDP (2015) 3D cell culture systems: advantages and applications. J Cell Physiol 230(1):16–26
5. Hofer M, Lutolf MP (2021) Engineering organoids. Nat Rev Mater 6(5):402–420
6. Watanabe N, Santostefano KE, Yachnis AT, Terada N (2017) A pathologist's perspective on induced pluripotent stem cells. Lab Invest 97(10):1126–1132
7. Moysidou C-M, Barberio C, Owens RM (2021) Advances in engineering human tissue models. Front Bioeng Biotechnol 8:620962
8. Velasco V, Shariati SA, Esfandyarpour R (2020) Microtechnology-based methods for organoid models. Microsyst Nanoeng 6(1):1–13
9. Kimura H, Sakai Y, Fujii T (2018) Organ/body-on-a-chip based on microfluidic technology for drug discovery. Drug Metab Pharmacokinet 33(1):43–48
10. Coluccio ML, Perozziello G, Malara N, Parrotta E, Zhang P, Gentile F et al (2019) Microfluidic platforms for cell cultures and investigations. Microelectron Eng 208:14–28
11. Kim JA, Hong S, Rhee WJ (2019) Microfluidic three-dimensional cell culture of stem cells for high-throughput analysis. World J Stem Cells 11(10):803–816
12. Magno V, Meinhardt A, Werner C (2020) Polymer hydrogels to guide organotypic and organoid cultures. Adv Funct Mater 30(48):2000097
13. Creff J, Malaquin L, Besson A (2021) In vitro models of intestinal epithelium: toward bioengineered systems. J Tissue Eng 12:2041731420985202
14. Kasendra M, Tovaglieri A, Sontheimer-Phelps A, Jalili-Firoozinezhad S, Bein A, Chalkiadaki A et al (2018) Development of a primary human Small Intestine-on-a-Chip using biopsy-derived organoids. Sci Rep 8(1):2871
15. Yu F, Hunziker W, Choudhury D (2019) Engineering microfluidic organoid-on-a-chip platforms. Micromachines 10(3):165
16. Grebenyuk S, Ranga A (2019) Engineering organoid vascularization. Front Bioeng Biotechnol 7:39
17. Kang S, Park SE, Huh DD (2021) Organ-on-a-chip technology for nanoparticle research. Nano Converg 8(1):20
18. Kaushik G, Gupta K, Harms V, Torr E, Evans J, Johnson HJ et al (2020) Engineered perineural vascular plexus for modeling developmental toxicity. Adv Healthc Mater 9(16):e2000825

19. Rawal P, Tripathi DM, Ramakrishna S, Kaur S (2021) Prospects for 3D bioprinting of organoids. BioDes Manuf 4(3):627–640
20. Drost J, Clevers H (2018) Organoids in cancer research. Nat Rev Cancer 18(7):407–418
21. Birgersdotter A, Sandberg R, Ernberg I (2005) Gene expression perturbation in vitro—a growing case for three-dimensional (3D) culture systems. Semin Cancer Biol 15(5):405–412
22. Giobbe GG, Crowley C, Luni C, Campinoti S, Khedr M, Kretzschmar K et al (2019) Extracellular matrix hydrogel derived from decellularized tissues enables endodermal organoid culture. Nat Commun 10(1):5658
23. Qian X, Jacob F, Song MM, Nguyen HN, Song H, Ming G-L (2018) Generation of human brain region-specific organoids using a miniaturized spinning bioreactor. Nat Protoc 13(3):565–580
24. Lancaster MA, Renner M, Martin C-A, Wenzel D, Bicknell LS, Hurles ME et al (2013) Cerebral organoids model human brain development and microcephaly. Nature 501(7467):373–379
25. Romero-Morales AI, O'Grady BJ, Balotin KM, Bellan LM, Lippmann ES, Gama V (2019) Spin∞: an updated miniaturized spinning bioreactor design for the generation of human cerebral organoids from pluripotent stem cells. HardwareX 6:e00084
26. Przepiorski A, Sander V, Tran T, Hollywood JA, Sorrenson B, Shih J-H et al (2018) A simple bioreactor-based method to generate kidney organoids from pluripotent stem cells. Stem Cell Rep 11(2):470–484
27. Haisler WL, Timm DM, Gage JA, Tseng H, Killian TC, Souza GR (2013) Three-dimensional cell culturing by magnetic levitation. Nat Protoc 8(10):1940–1949
28. Souza GR, Molina JR, Raphael RM, Ozawa MG, Stark DJ, Levin CS et al (2010) Three-dimensional tissue culture based on magnetic cell levitation. Nat Nanotechnol 5(4):291–296
29. Ferreira JN, Hasan R, Urkasemsin G, Ng KK, Adine C, Muthumariappan S et al (2019) A magnetic three-dimensional levitated primary cell culture system for the development of secretory salivary gland-like organoids. J Tissue Eng Regen Med 13(3):495–508
30. Casson J, O'Kane S, Smith C-A, Dalby M, Berry C (2018) Interleukin 6 plays a role in the migration of magnetically levitated mesenchymal stem cells spheroids. Appl Sci (Switzerland) 8:412
31. Kundu J, Shim J-H, Jang J, Kim S-W, Cho D-W (2015) An additive manufacturing-based PCL-alginate-chondrocyte bioprinted scaffold for cartilage tissue engineering. J Tissue Eng Regen Med 9(11):1286–1297
32. Nguyen D, Hägg DA, Forsman A, Ekholm J, Nimkingratana P, Brantsing C et al (2017) Cartilage tissue engineering by the 3D bioprinting of iPS cells in a nanocellulose/alginate bioink. Sci Rep 7(1):658
33. Tong A, Pham QL, Abatemarco P, Mathew A, Gupta D, Iyer S et al (2021) Review of low-cost 3D bioprinters: state of the market and observed future trends. SLAS Technol 26(4):333–366
34. Kapałczyńska M, Kolenda T, Przybyła W, Zajączkowska M, Teresiak A, Filas V, et al (2016) 2D and 3D cell cultures – a comparison of different types of cancer cell cultures. Aaoms. https://www.termedia.pl/doi/10.5114/aoms.2016.63743
35. Edmondson R, Broglie JJ, Adcock AF, Yang L (2014) Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol 12(4):207–218
36. Chen F-M, Liu X (2016) Advancing biomaterials of human origin for tissue engineering. Prog Polym Sci 53:86–168
37. Ivanov DP, Parker TL, Walker DA, Alexander C, Ashford MB, Gellert PR et al (2014) Multiplexing spheroid volume, resazurin and acid phosphatase viability assays for high-throughput screening of tumour spheroids and stem cell neurospheres. PLoS One 9(8):e103817
38. Shi W, Kwon J, Huang Y, Tan J, Uhl CG, He R et al (2018) Facile tumor spheroids formation in large quantity with controllable size and high uniformity. Sci Rep 8(1):6837
39. Zhu J, Marchant RE (2011) Design properties of hydrogel tissue-engineering scaffolds. Expert Rev Med Devices 8(5):607–626

40. Białkowska K, Komorowski P, Bryszewska M, Miłowska K (2020) Spheroids as a type of three-dimensional cell cultures-examples of methods of preparation and the most important application. Int J Mol Sci 21(17):E6225
41. Huang S-W, Tzeng S-C, Chen J-K, Sun J-S, Lin F-H (2020) A dynamic hanging-drop system for mesenchymal stem cell culture. Int J Mol Sci 21(12):4298
42. Bartosh TJ, Ylostalo JH (2014) Preparation of anti-inflammatory mesenchymal stem/precursor cells (MSCs) through sphere formation using hanging drop culture technique. Curr Protoc Stem Cell Biol 28:Unit-2B.6
43. Lu H, Tang S-Y, Yun G, Li H, Zhang Y, Qiao R et al (2020) Modular and integrated systems for nanoparticle and microparticle synthesis—a review. Biosensors 10(11):165
44. Chaicharoenaudomrung N, Kunhorm P, Noisa P (2019) Three-dimensional cell culture systems as an in vitro platform for cancer and stem cell modeling. World J Stem Cells 11(12):1065–1083
45. Kolehmainen K, Willerth SM (2012) Preparation of 3D fibrin scaffolds for stem cell culture applications. J Vis Exp 61:e3641
46. Nikolova MP, Chavali MS (2019) Recent advances in biomaterials for 3D scaffolds: a review. Bioact Mater 4:271–292
47. Xu C, Lu W, Bian S, Liang J, Fan Y, Zhang X (2012) Porous collagen scaffold reinforced with surfaced activated PLLA nanoparticles. ScientificWorldJournal 2012:695137
48. Chen S, Zhang Q, Nakamoto T, Kawazoe N, Chen G (2016) Gelatin scaffolds with controlled pore structure and mechanical property for cartilage tissue engineering. Tissue Eng Part C Methods 22(3):189–198
49. Afewerki S, Sheikhi A, Kannan S, Ahadian S, Khademhosseini A (2018) Gelatin-polysaccharide composite scaffolds for 3D cell culture and tissue engineering: towards natural therapeutics. Bioeng Transl Med 4(1):96–115
50. Ferreira BMP, Andersson N, Atterling E, Engqvist J, Hall S, Dicko C (2020) 3D structure and mechanics of silk sponge scaffolds is governed by larger pore sizes. Front Mater 7:211
51. Lim JI, Im H, Lee W-K (2015) Fabrication of porous chitosan-polyvinyl pyrrolidone scaffolds from a quaternary system via phase separation. J Biomater Sci Polym Ed 26(1):32–41
52. Chen C-Y, Ke C-J, Yen K-C, Hsieh H-C, Sun J-S, Lin F-H (2015) 3D porous calcium-alginate scaffolds cell culture system improved human osteoblast cell clusters for cell therapy. Theranostics 5(6):643–655
53. Xu H, Jiao Y, Qin S, Zhao W, Chu Q, Wu K (2018) Organoid technology in disease modelling, drug development, personalized treatment and regeneration medicine. Exp Hematol Oncol 7:30
54. Li Y, Tang P, Cai S, Peng J, Hua G (2020) Organoid based personalized medicine: from bench to bedside. Cell Regen 9:21
55. Murrow LM, Weber RJ, Gartner ZJ (2017) Dissecting the stem cell niche with organoid models: an engineering-based approach. Development 144(6):998–1007
56. Yin X, Mead BE, Safaee H, Langer R, Karp JM, Levy O (2016) Stem cell organoid engineering. Cell Stem Cell 18(1):25–38
57. Frum T, Spence JR (2021) hPSC-derived organoids: models of human development and disease. J Mol Med (Berl) 99(4):463–473
58. Jin RU, Mills JC (2020) Tumor organoids to study gastroesophageal cancer: a primer. J Mol Cell Biol 12(8):593–606
59. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X (2020) Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal Transduct Target Ther 5(1):1–23
60. Hyvärinen T, Hyysalo A, Kapucu FE, Aarnos L, Vinogradov A, Eglen SJ et al (2019) Functional characterization of human pluripotent stem cell-derived cortical networks differentiated on laminin-521 substrate: comparison to rat cortical cultures. Sci Rep 9(1):17125
61. Bartfeld S, Bayram T, van de Wetering M, Huch M, Begthel H, Kujala P et al (2015) In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. Gastroenterology 148(1):126–136.e6

62. Dahl-Jensen S, Grapin-Botton A (2017) The physics of organoids: a biophysical approach to understanding organogenesis. Development 144(6):946–951
63. Muffat J, Li Y, Yuan B, Mitalipova M, Omer A, Corcoran S et al (2016) Efficient derivation of microglia-like cells from human pluripotent stem cells. Nat Med 22(11):1358–1367
64. Park J, Wetzel I, Marriott I, Dréau D, D'Avanzo C, Kim DY et al (2018) A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. Nat Neurosci 21(7):941–951
65. Malhotra N, Lee J-S, Liman RAD, Ruallo JMS, Villaflores OB, Ger T-R et al (2020) Potential toxicity of iron oxide magnetic nanoparticles: a review. Molecules 25(14):3159

Liver-on-a-Chip

15

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Abstract

Hepatotoxicity preclinical experiments using animal models have many limitations such as time consuming, expensive, and mismatching cross-species physiology that affects interpretation in predicting clinical outcome. Organ-on-a-chip (OoC) is a cutting-edge technology that helps to mimic the microarchitecture and reproduce physiological functions of an organ in vitro cell-based model system. Organ-on-a-chip representing skin, brain, liver, lung, kidney, etc. has been explored for modeling the physiological functions in vitro. These microfluidic-based devices harbor functionally active cells in two- or three-dimensional culture platform to recapitulate the fluid dynamics and physiological capacities. The liver-on-a-chip (LoC) is one such potential device that suits the necessity in the field of drug designing and toxicity screening studies. LoC also performs as a platform of in vitro disease models to explore toxicity and pharmacokinetic analysis of drugs. There are different microfluidic designs for LoC composed of cell compartment that interact with extracellular matrix to form the cytoarchitecture and physiological flow that enables long-term cell culture and heterotypic cell–cell interactions. LoCs connected to other organ representatives like gut-liver model, lung-liver model, and kidney-liver-lung model form multiorgan-on-a chip that allows insight to cross-organ interaction and could help in studying various systemic diseases.

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Bile · Cyp450 · DILI · Hepatocytes · HepG2 · Liver-on-a-chip · Microfluidics · Sinusoids

15.1 Introduction

The drug screening tests or any biological assessment of toxicity are done on in vitro cultured cells in labs and in animal trials. Despite the fact that animal experiments are the most reliable way of pharmacological evaluation, only a small percentage of drugs makes it through Phase I clinical trials and onto the market [1]. In addition to these constraints, post-market drug withdrawal is another terrible situation that pharmaceutical companies are concerned about [2]. This is due to untrustworthy predictions arrived at from the in vivo data. In vitro methods have been thoroughly explored as a preliminary risk analysis approach to avoid the catastrophic scenario of drug withdrawal. Advanced in vitro culture systems and tiny devices have also been thoroughly researched for this purpose. Organ-on-a-chip is one of most advanced emerging technologies for maintaining cells by recreating the intricate microenvironment of human tissues and organs in vitro. Organs-on-a-chip are miniature devices that mimic miniaturized functional units of human organ systems using a mix of technologies such as cell biology, microfabrication engineering, and biomedical technology. The miniaturized microfluidic system can accommodate single or multiple cell types of a tissue or organ, will employ the fluid flow for efficient mass transfer, and creates cell–cell interfaces of a tissue. Sin et al. described "microscale cell culture analogs" as the microfluidic system with interconnected representative cells from different organs that are recirculated with tissue culture medium to meet the multi-organ complex cell interactions [3]. The organ-on-a-chip also represents complex microenvironment of cells within a tissue to get physiological readout in a higher resolution. The microfluidic system models miniaturized in vitro are alternatives to animal testing by reducing the size to equivalents in the order of 1/100,000th scale of the organ [4].

15.1.1 Liver-on-a-Chip Device

Liver-on-a-chip is a microfluidic device that recreates in vitro three-dimensional (3D) liver micro-physiological conditions on a microscopic scale. The device should be adaptable to high throughput and also mimics the microarchitecture of liver and its dynamic physicochemical environment. The advantages of liver-on-a-chip devices span over many disciplines of medical application such as drug discovery, biochemical aspects in biotransformation, mechanism and progression of liver diseases, hormonal effects on organ system, inhibition and activation of metabolic systems, in vitro toxicology, and personalized medicine.

15.1.2 Parenchymal and Non-parenchymal Cells

The cell types in liver can be categorized as parenchymal and non-parenchymal compartments. The main features of both categories of cells are summarized in Table 15.1 [5]. The primary and the most abundant cell type are the highly differentiated hepatocytes that provide all the functions that are of liver as an organ. The main functions of hepatocytes are metabolism of internal and external substance through Phase I and Phase II enzymes, albumin synthesis, urea production, and glycogen storage. The metabolic capabilities of hepatocytes are not distributed evenly throughout the liver, but are arranged in various zones of liver lobules [6]. Liver sinusoidal endothelial cell (LSEC) lines contribute to around 50% of non-parenchymal cells. Unlike typical epithelial cells or other vascular endothelial cells, LSECs lack a basement membrane and are highly fenestrated [7]. Hepatic stellate cells are also called as perisinusoidal cells or fat-storing cells based on the location where it is found and its peculiar function. It occurs in non-activated and activated stage and plays a major role in liver fibrosis. Kupffer cells are resident macrophages in liver that form around 15% of non-parenchymal liver cells. They are found attached to sinusoidal endothelial surface and phagocyte foreign particulate matters that enter into liver through blood [8]. Kupffer cells release immunomodulatory molecules such as tumor necrosis factor-α, interleukin-1, and Interleukin-6. The cells that are present in the bile duct wall in the liver are biliary epithelial cells. These cells are approximately 10 μm in size and are not having any major liver functions other than their minor role in bile excretion pathway [9]. Some of the key features of the liver cells are given in Table 15.1.

15.1.3 Role of Cells in Drug Liver Metabolism and Toxicity

Toxicological studies related to liver require functional hepatocytes that can be used for the in vitro analysis. However, the primary hepatocytes dedifferentiate rapidly as soon as they are removed from the in vivo environment. The crucial hepatic properties such as albumin synthesis, urea production, and cytochrome P450 (CYP450) activity will be affected making it useless for the purpose of study [10]. The drug screening in animals is the unavoidable expensive and time-consuming process in the pharmaceutical industry. Such animal testing sometimes is misleading due to difference in species and normal liver physiology resulting in drug-induced liver injury (DILI) and subsequent withdrawal of drug from the market after primary approval [11]. The inaccuracy in reflecting the human conditions in animal models is due to the interspecies differences in expression of genes related to drug absorption, distribution, metabolism, and excretion [12, 13]. Hence, in vitro cell models are advocated globally for a preliminary screening of drugs before animal trials. However, despite its many advantages like high throughput, being economical, and sensitivity to cellular level, in vitro models are far away from the in vivo conditions of liver due to lack of three-dimensional architecture and fluid flow conditions. The in vitro systems are relatively low cost if hepatic cell lines such

Table 15.1 Comparison and features of parenchymal and non-parenchymal cells of liver

	Parenchymal cells	Non-parenchymal cells			
Features	Hepatocytes	Kupffer cells	Stellate cells	Liver sinusoidal endothelial cells	Biliary epithelial cells
Morphology (illustration)					
Cell type	Epithelial	Macrophage	Fibroblastic	Epithelial	Epithelial
Size (μm)	20–30	10–13	11–12	6–11	10
Representation (%)	60–65	15	8	16	<5
Characteristics	Largest cells; metabolically active; functional; glycogen storage; binucleated	Motile; irregular in shape; secretion of inflammatory molecules	Vitamin-storing; collagen deposition	Fenestrated cells; filtration; immune response	Synthesis of proteoglycans and glycoproteins

as HepG2, Hep3B, or HepaRG are used. These cancerous cells also lack 3D environment and eventually the levels of drug-metabolizing enzymes are downregulated [14]. The bridge between the in vitro and in vivo conditions can be achieved if the arrangement of multiple cell types is in an anatomically relevant manner to bring appropriate cellular communications within a simulated fluid flow under shear force [15]. In vitro studies using human primary hepatocytes are thus very important to understand the metabolism, fate of drug, and resulting toxicity in humans prior to clinical trials [16]. Though the primary human hepatocytes have been considered to be the ideal cells for in vitro models, almost 50% of the liver-specific activities are lost during in vitro culture [14]. Therefore, in vitro culture models have to be developed to simulate the 3D microenvironment to maintain the primary hepatocyte functions for a longer duration of several weeks. Efforts on establishing reliable in vitro liver models for studying the physiological and pathological responses toward drugs and molecules include two-dimensional hepatocyte monolayer, co-culture of parenchymal with non-parenchymal cells, perfused cell culture, 3D culture systems, liver organoids, and liver-on-a-chip systems [17–20].

15.2 In Vitro Chip Models

The organs-on-a-chip are miniaturized microfluidic devices that encompass living cells representing various organs and replicate multi-organ physiology in an in vitro platform. It is defined as, "the devices for culturing living cells in continuously perfused, micrometer-sized chambers in order to model physiological functions of tissues and organs" [21, p. 766]. Thus, organs-on-a-chip are effective micro-physiological systems (MPS) that are capable to provide information during various stages of the drug screening process [22]. Low et al. explained tissue chips as: ". . .microphysiological systems (MPS), are devices designed to position cells in a three-dimensional structure that mimic the function of organs of the body, and react in a physiological manner to exposure to drugs, hormones, cell signaling molecules and biomechanical stressors" [23, p. 3026].

15.2.1 Major Constituents of Organ-on-a-Chip

The continents of the liver-on-a-chip are the microfluidic device, cell compartment (one cell or co-culture of different cells), perfusion system, and environment control (extracellular matrix coating, temperature, and pH).

15.2.2 Materials for Fabrication of Microfluidic Device

The basic component that is essential for the development of liver-on-a-chip microfluidic device is the material used for the device fabrication. The material should cater to many properties such as cell compatibility, chemical compatibility,

thermostability, pressure resistance, transparency, and non-toxicity. Devices are fabricated from aluminum, copper, and iron with properties such as chemical resistance [24]. Silicon has been widely proposed for the microfluidic fabrication due to the ready availability, chemical compatibility, and thermostability. However, the fragile nature, high elastic modulus, and opacity of silicon pose a limitation that renders it not suitable for optical detection in certain wavelengths of light when used for liver-on-a-chip device with detection [25]. Another commonly used material in microfluidic device is glass, due to the transparent property along with characteristics like chemical inertness, thermostability, being electrically insulating, biologically compatible, and having option for surface functionalization [26]. Another well-accepted and widely used material for fabrication of liver-on-a-chip is the polymers, placing it above silicon and glass. Polymers are less expensive than organic materials, metals, and glass [25]. Devices can be manufactured easily and at a lower cost with lot of flexibility in modification. Additionally, polymers have positive characteristics such as high temperature stability (up to 200 °C), they can be mass manufactured, are optically clear, are non-toxic, have low autofluorescence, hydrophobicity, high elasticity, and many other special properties. Some of the widely used polymers used in lab and at industrial scale are polydimethylsiloxane (PDMS), polymethylmethacrylate, fluoropolymers, thiol-ene polymers (TEs), etc. [26] Other materials used for microfluidic devices are ceramics, resins, hydrogels, paper, and hybrid materials using the above for specific purpose [27].

15.2.3 Cells in Liver-on-a-Chip Device

The main biological components in liver-on-a-chip device are the cells, especially hepatocytes, which represent the whole functional compartment of the liver. The hepatocytes used in such devices need to be functional and while using in the microfluidic environment the cells need to be viable for a longer duration. Three major types of cells used within in vitro hepatotoxicity models are primary human hepatocytes, liver-derived cell lines, and stem-cell-derived hepatocytes. While primary hepatocytes are gold standard with regard to liver functions, they are also very difficult to maintain due to dedifferentiation under culture conditions. Hence the device should support the major liver functions such as albumin synthesis, ammonia detoxification, phase I and II metabolic enzyme activities, and glucose metabolism. The most widely used hepatic cell lines for in vitro screening are HepG2, Huh7, Hep3B, and SK-Hep-1 [28]. Though the cell lines do not represent all the liver functions expressed by the primary hepatocytes, they have unlimited proliferation and a stable phenotype making it widely used for toxicological studies. Cells lines sometimes give inaccurate responses due to low metabolic enzyme activities and low expression of transport proteins. An alternative to cell lines are stem-cell-derived hepatocytes that have advantage of unlimited proliferation in vitro and at the same time achieve required hepatocyte functions. Hepatocyte-like cells differentiated from induced pluripotent stem cells (iPSC) express functional hepatic activity such as urea

and albumin [29]. However, iPSC-derived hepatocyte-like cells also express fetal marker known as alpha fetoprotein and reduced activity of drug metabolizing cytochrome p450. The microfluidic device performs like a micro-bioreactor. The hepatocytes are cultured within the device as monolayer, spheroids, sandwiched in gels or scaffolds, or as entrapped within hydrogels.

15.2.4 Other Supporting Accessories

The durability of cells in microfluidic devices to maintain the cell viability and function is to connect it to a passive or pressure-driven perfusion system [30]. The perfusion shall be of recirculating or non-recirculating nature, depending on the liver-on-a-chip model. Recirculation system is used when the paracrine effect is studied and non-recirculating perfusion is selected when cell–cell communication is not important. Many physical parameters such as bubble formation, temperature variation, and shear stress should be addressed using an appropriate perfusion apparatus.

15.3 Different Models of Liver-on-a-Chip

15.3.1 Liver-on-a-Chip Based on 2D Planar Culture

Conventional monolayer culture is relatively easy to practice and used for high-throughput screening. The loss of hepatocyte functions shall be prevented by co-culture with non-parenchymal cells. The cells in monolayer are devoid of 3D environment and lack cell polarization.

15.3.2 Liver-on-a-Chip Based on 3D Spheroids

Three-dimensional (3D) culture systems are considered to be more functional compared to the conventional 2D culture systems. Cell aggregates also called as cell spheroids replicate functions of the tissue and are structurally organized. Three-dimensional microtissues such as spheroids achieve extensive cell–cell contacts and while in co-culture maintain hepatocellular phenotype and the differentiated functions. Hanging drop culture creates microtissues that are useful for high-throughput screening [31]. Spheroid cultures are supposed to increase the in vitro functional maintenance and when they are included within a microfluidic system, they further improve the structural, functional, and the life span of spheroids in vitro [32]. Hence, including 3D spheroids from liver cells in the microfluidic system will be an added advantage to liver-on-a-chip devices. Spheroids can be homotypic (one cell type) or heterotypic (more than one cell) in nature. Lee et al. developed liver-on-a-chip device to study the interaction of hepatocytes and hepatic stellate cells [33]. The hepatocyte spheroids were co-cultured with stellate cells without direct

cell–cell contact and it was found that the paracrine effect on hepatocyte spheroids improved liver-specific functions. Once the spheroid is available, it can be incorporated into any form of liver-on-a-chip devices. For instance, Bhise et al. reported liver-on-a-chip platform for culturing HeG2/C3A spheroids [34]. The spheroids embedded in hydrogel were loaded into chambers of microfluidic device fabricated using polydimethylsiloxane (PDMS). The engineered construct was functional and expressed hepatocyte markers such as cytokeratin 18, multidrug resistance protein 2, bile canalicular protein, and tight junction protein.

15.3.3 Liver-on-a-Chip Based on Layer-by-Layer Deposition

Three-dimensional printing is a layer-by-layer additive manufacturing technology that is developing rapidly due to its potential application in all disciplines including medical science. This rapid prototyping method has been employed in fabrication of microfluidic chips that would help to reduce the translation time from concept to reality when compared to very complex multistep process of photolithographic techniques [35]. Cells and extracellular matrix are laid out according to a preset path through a 3D printer in the form of additive liver-on-a-chip based on matrix-dependent 3D culture [36]. Three-dimensional bioprinting technology allows construction of complex biological structures using various cell types and biomaterials in a high throughput manner. Though time saving, 3D Bioprinting is limited by the difficulty in positioning individual cells in the construct and the lack of through optimization of bioprinted materials.

15.3.4 Liver-on-a-Chip-Based Microarrays

Micropatterning of cells to achieve controlled migration, cell morphology, and function can be a powerful tool for high-throughput cell-based bioassays [37]. Liver-on-a-chip-based cell microarrays in micro well plates have advantage over normal microfluidic liver-on-a-chip. However, lack of spatial distribution and cellular interactions of cells in vivo might not be possible in such system as cells are specifically attached to a substratum. This system might be useful to study the paracrine effects of cells in a tissue.

15.3.5 Microfluidic Hepatic Lobule

The lobule represents the functional unit of the liver. Some of the liver-on-a-chip models try to simulate the sinusoids, the lobule, and zonation of lobule. Chao Ma et al. reported 3D liver-lobule-like microtissue that maintained higher levels of cytochrome p450 and UGT activities [38]. In order to mimic liver lobule, microphysiological niche for hepatocytes similar from portal triad to central vein is required. A very large-scale liver-lobule-on-a-chip device was reported by

Banaeiyan et al. [39]. The device consisted of hexagonal chambers that connect to a central vein mimicking the fluid flow in lobule (Table 15.2). Ho et al. developed a lobule-mimetic cell-patterning technique using an enhanced field-induced dielectrophoresis to simulate hepatic lobule. The randomly dispersed hepatocytes and endothelial cells inside the microfluidic device separately aligned into predetermined patterns to mimic the lobule [40]. The metabolic capability of such system was higher compared to cells in monolayer and non-patterned systems.

15.3.6 Microfluidic Zonation of the Lobule

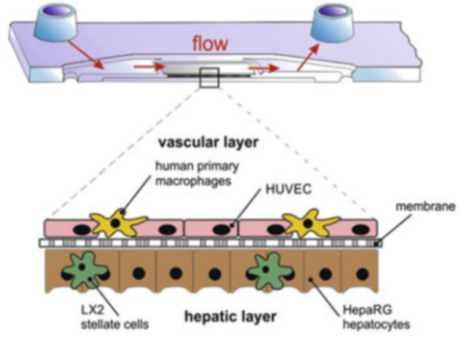
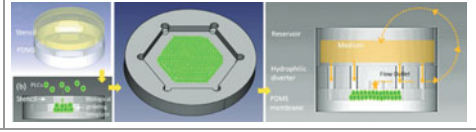
The lobule is highly heterogeneous with respect to the metabolic activities across the liver sinusoid. Such metabolic zonation was modeled by Kang et al. in a metabolic-patterning-on-a-chip (MPOC) device [41]. Gradients in nitrogen, carbohydrate, and xenobiotic metabolism were enforced across the length of the tissue to create in-vivo-like zonation.

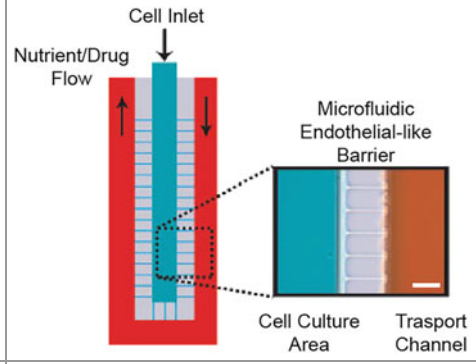
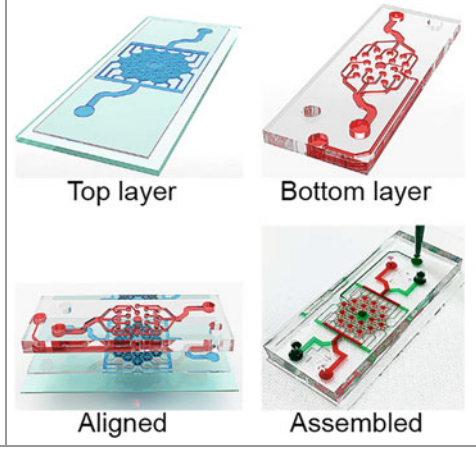
15.3.7 Microfluidic Hepatic Sinusoid

Liver sinusoid represents the main functional site in the liver containing parenchymal and non-parenchymal cells. Simulating such a complex arrangement of different types of cells confined to a microscale dimension is possible only by microfabrication techniques. Du et al. developed two-layer channel device by micro-engineering technique, where the channels were connected by a membrane [42]. The sinusoids and the interstitial fluid flow in the space of Disse were recreated by incorporating shear flow into the channels. Oxygen concentration is a critical parameter in sinusoidal blood flow. Ya et al. developed a self-assembled perfusable hepatic sinusoid network named as “lifelike bionic liver lobule chip” for higher metabolic abilities for longer duration [42]. Lee et al. developed an artificial barrier between primary hepatocytes and endothelial-like barrier from a convective transport vessel. The design maintained hepatocyte function over 7 days without extracellular matrix coating and responded to drugs in dose-dependent manner [43]. In addition to creating physical compartmentalization of cells in sinusoids, for better understanding of dissolved oxygen in a cell culture an analog device was fabricated using silicon [3]. An oxygen sensor based on ruthenium complex was incorporated into the system, which functioned as real-time sensors into a perfused system. The three-chamber microculture analog device replicated the liquid residence time parameters of pharmacokinetic model.

Some of the liver-on-a-chip devices reported for various purposes are tabulated in Table 15.2.

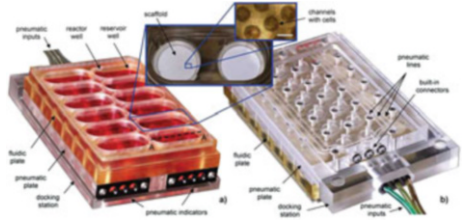
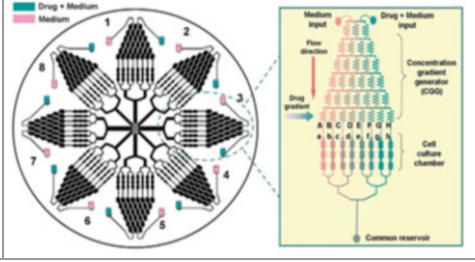
Table 15.2 Liver-on-a-chip devices showing different designs and purpose. (Representative figures are adopted from the cited references with permission)

Cells	Cell polarity	Time (weeks)	Characteristics	Illustration	Reference
HepaRG cells; NPCs	Yes	<1	CYP450(+); bile canaliculi-like structures; recapitulates physiological function		[44]
Primary rat hepatocytes; HSCs	No	2	Multiscale structural hierarchy in a scaffold-free condition; does not recapitulate full function		[45]

HepG2/hiCell	Yes	2–3	Scalable; functional bile-canaliculi network in hiCell hepatocytes		[43]
Rat and human primary hepatocyte	No	1	Sustaining of hepatocytes without an ECM coating		[39]

(continued)

Table 15.2 (continued)

Cells	Cell polarity	Time (weeks)	Characteristics	Illustration	Reference
Rat primary hepatocyte; NPC	Yes	2	Generation of 3D microscale tissue units in hundreds; recapitulates physiological function		[46]
HepG2 cells	Yes	24 h	High content screening of cells; contains integrated gradient generators for toxicity tests		[47]

15.3.8 Liver-on-a-Chip Disease Models

Drug-induced liver injury (DILI) is of primary concern when it comes to drug development, which is poorly understood. The main reasons involved include the species differences in liver toxicity and the limited predictivity of conventional in vitro models. The liver-on-a-chip is an in vitro microfluidic culture device that contains continuously perfused chambers inhabited by cells, which in turn recapitulate the liver microenvironment [21]. The chip in its own creates a physiologically relevant cell culture microenvironment that could mimic the in vivo environment of the liver, which is not attained during standard plate culture [48]. Some of the microfluidic systems described above also represent the liver disease models. Certain specific liver and other tissue models are described below.

A demountable liver-sinusoid-on-a-chip device for study of alcoholic liver disease (ALD) was proposed by Deng et al. The device incorporates HepG2, LX-2, EAhy926, and U937 cells and serves for investigating the pathophysiological system of individual non-parenchymal cells in alcohol-induced ALD [49]. Similarly, a spheroid-based microfluidic chip was developed as an in vitro model to study ALD progression [16]. Another disease model is the sequentially layered, self-assembly liver (SQL-SAL) model using primary human hepatocytes along with human endothelial (EA.hy926), immune (U937), and stellate (LX-2) cells in physiological ratios and is able to maintain major physiological functions for at least 28 days [36]. Hepatocellular carcinoma has been ranked as the disease that accounts for the third highest cancer-related death globally, which indicates the need for early diagnosis of non-alcoholic fatty liver disease (NAFLD) as a potential risk factor. A human in vitro test model was developed within a microfluidic device in a sinusoid-like fashion, forming a more permissive tissue-like microenvironment for understanding the NAFLD pathogenesis [50]. Liver-on-a-chip device can also give information on interaction of tumor cells and metastatic sites. Kim et al. reported a liver-on-a-chip 3D microfluidic system that creates a premetastatic niche for the breast-cancer-derived extracellular vesicles [51]. Experiments with the device gave insights into endothelial to mesenchymal transition.

15.3.9 Multi-organ Models

Multi-organ chip devices allow collection of information of multiple organ types to study the safety and efficacy of materials and chemicals.

15.3.9.1 Liver–Lung Model

The effect of inhaled compounds by lung on liver can be studied on a multi-organ liver-on-a-chip model. The liver–lung model will help in evaluation of metabolized compounds in liver and also to know the effect of inhaled compounds in the blood. David et al. developed a lung/liver-on-a-chip model to investigate the possible toxicity of inhaled chemicals [52]. It is made up of a single circuit, normal human bronchial epithelial (NHBE) cells cultured at the air–liquid interface (ALI), and

HepaRGTM liver spheroids that were co-cultured in the chip for 28 days and remained viable and functioning. It can also be used to test other substances that are given in a different way such as mimicking the inhalation route.

15.3.9.2 Liver–Gut Model

The in vitro drug screening with single-cell culture does not reflect the sequence of drug transformation that would happen in vivo. Hence, the effects of side effects and first-pass metabolism are completely lacking in conventional single-cell test methods. After oral intake of drugs, drugs go through the first-pass metabolism in the gut and the liver, which greatly affects the final outcome of the drugs' efficacy and side effects. The first-pass metabolism is a complex process involving the gut and the liver tissue, with transport and reaction occurring simultaneously at various locations, which makes it difficult to be reproduced in vitro with conventional cell culture systems. Choe et al. reported a gut–liver co-culture microfluidic test system with gut epithelium and hepatocytes to understand the first-pass metabolism of flavonoid [53]. The function of gut and liver compartments of the model showed a change, with increased absorptive property in Caco-2 cells and enhanced cytochrome P450 metabolic activity in both the cell types. A pharmacokinetic chip model was reported by Lee et al., who used 3D gut–liver chip for prediction of first-pass metabolism [54]. It was observed that the gut–liver co-culture chip design slowed the drug clearance relative to cell culture area or cell volume indicating that the chip forms a platform to study the first-pass metabolism of drugs.

15.3.9.3 Liver–Kidney–Lung Model

Cell culture systems with compartmentalization of multi-organs have immense application in drug screening [55]. A PDMS chip comprising of fat, kidney, liver, and lung cells, called the 3D microfluidic cell culture system (3D-μFCCS), was reported by Zhang et al. An array of micropillars was designed, surrounding a central cell culture chamber with two medium streams flow around the array with diffusion of medium from cell compartments.

15.3.9.4 Liver–Testis Model

The effect of toxicants on human health and reproduction needs extensive preliminary screening before going to animal trials. Assessment of reproductive risk and accurate predication of testicular toxicity can be modeled in vitro. A co-culture system with liver cells or liver equivalent will help to understand more on the biotransformation effect and would improve reprotoxicity forecast in humans. Such kind of functionally interactive co-culture of liver and testis equivalents was reported by Baert et al. [56] The testicular organoids were co-cultured in a multi-organ-chip that was connected to a microfluidic stream containing liver spheroid-specific medium. The study gave insights into the hormonal release by one organ system and influence of cytochrome P450 enzyme system of the counterpart.

15.4 Conclusions

Microfluidic technology has undoubtedly revolutionized the toxicology testing. Liver-on-a-chip device has established a standalone advantage in biomedical research by bridging the gap between conventional static cell culture and in vivo animal experiments. Possibility of complex designing, advancement of microfabrication techniques, and the data available from last few decades of research indicate that, in future, liver-on-a-chip technology is going to be in the routine evaluation to meet regulatory needs. There are still more research and development required in the present models to address innervation, vasculature, and addition of immune components into the device. More practical supporting devices such portable perfusion systems, incubators, and bioreactors need to be developed to support the growth of liver-on-a-chip device development to make it high throughput. It is necessary to combine liver-on-a-chip technology with biosensing devices for non-invasive assessment of cell viability, proliferation, and functions.

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References

1. Zou D, Cui D (2018) Advances in isolation and detection of circulating tumor cells based on microfluidics. Cancer Biol Med 15:335–353
2. Pritchard JF, Jurima-Romet M, Reimer MLJ, Mortimer E, Rolfe B, Cayen MN (2003) Making better drugs: decision gates in non-clinical drug development. Nat Rev Drug Discov 2:542–553
3. Sin A, Chin KC, Jamil MF, Kostov Y, Rao G, Shuler ML (2004) The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors. Biotechnol Prog 20:338–345
4. Maschmeyer I, Hasenberg T, Jaenicke A indicate (2015) Chip-based human liver–intestine and liver–skin co-cultures – a first step toward systemic repeated dose substance testing in vitro. Eur J Pharm Biopharm 95:77–87
5. Deng J, Cong Y, Han X et al (2020) A liver-on-a-chip for hepatoprotective activity assessment. Biomicrofluidics 14:064107
6. Treyer A, Müsch A (2013) Hepatocyte polarity. Compr Physiol 3:243–287
7. Poisson J, Lemoinne S, Boulanger C et al (2017) Liver sinusoidal endothelial cells: physiology and role in liver diseases. J Hepatol 66:212–227
8. Laskin DL (1990) Nonparenchymal cells and hepatotoxicity. Semin Liver Dis 10:293–304
9. Deng J, Zhang X, Chen Z et al (2019) A cell lines derived microfluidic liver model for investigation of hepatotoxicity induced by drug-drug interaction. Biomicrofluidics 13:024101
10. Usta OB, McCarty WJ, Bale S et al (2015) Microengineered cell and tissue systems for drug screening and toxicology applications: evolution of in-vitro liver technologies. Technology 3: 1–26
11. Fisher K, Vuppalanchi R, Saxena R (2015) Drug-induced liver injury. Arch Pathol Lab Med 139:876–887
12. Chapman KL, Holzgrefe H, Black LE et al (2013) Pharmaceutical toxicology: designing studies to reduce animal use, while maximizing human translation. Regul Toxicol Pharmacol 66:88–103

13. Kammerer S (2021) Three-dimensional liver culture systems to maintain primary hepatic properties for toxicological analysis in vitro. Int J Mol Sci 22(19):10214
14. Ware BR, Khetani SR (2017) Engineered liver platforms for different phases of drug development. Trends Biotechnol 35:172–183
15. Lauschke VM, Hendriks DF, Bell CC, Andersson TB, Ingelman-Sundberg M (2016) Novel 3D culture systems for studies of human liver function and assessments of the hepatotoxicity of drugs and drug candidates. Chem Res Toxicol 29:1936–1955
16. Khetani SR, Berger DR, Ballinger KR, Davidson MD, Lin C, Ware BR (2015) Microengineered liver tissues for drug testing. J Lab Autom 20:216–250
17. Choi WM, Eun HS, Lee YS et al (2019) Experimental applications of in situ liver perfusion machinery for the study of liver disease. Mol Cells 42:45–55
18. Tomlinson L, Hyndman L, Firman JW et al (2019) In vitro liver zonation of primary rat hepatocytes. Front Bioeng Biotechnol 7:17
19. Kim Y, Kang K, Jeong J et al (2017) Three-dimensional (3D) printing of mouse primary hepatocytes to generate 3D hepatic structure. Ann Surg Treat Res 92:67–72
20. Lancaster MA, Knoblich JA (2014) Organogenesis in a dish: modeling development and disease using organoid technologies. Science (New York, NY) 345:1247125
21. Bhatia SN, Ingber DE (2014) Microfluidic organs-on-chips. Nat Biotechnol 32:760–772
22. Low LA, Mummery C, Berridge BR, Austin CP, Tagle DA (2021) Organs-on-chips: into the next decade. Nat Rev Drug Discov 20:345–361
23. Low LA, Tagle DA (2017) Tissue chips - innovative tools for drug development and disease modeling. Lab Chip 17:3026–3036
24. James M, Revia RA, Stephen Z, Zhang M (2020) Microfluidic synthesis of iron oxide nanoparticles. Nanomaterials (Basel) 10:2113
25. Ren K, Zhou J, Wu H (2013) Materials for microfluidic chip fabrication. Acc Chem Res 46: 2396–2406
26. Nielsen JB, Hanson RL, Almughamsi HM, Pang C, Fish TR, Woolley AT (2020) Microfluidics: innovations in materials and their fabrication and functionalization. Anal Chem 92:150–168
27. Niculescu AG, Chircov C, Bîrcă AC, Grumezescu AM (2021) Fabrication and applications of microfluidic devices: a review. Int J Mol Sci 22(4):2011
28. Nikolic M, Sustersic T, Filipovic N (2018) In vitro models and on-chip systems: biomaterial interaction studies with tissues generated using lung epithelial and liver metabolic cell lines. Front Bioeng Biotechnol 6:1–13
29. Schwartz RE, Fleming HE, Khetani SR, Bhatia SN (2014) Pluripotent stem cell-derived hepatocyte-like cells. Biotechnol Adv 32:504–513
30. Hattori K, Sugiura S, Kanamori T (2014) Microfluidic perfusion culture. Methods Mol Biol (Clifton, NJ) 1104:251–263
31. Messner S, Agarkova I, Moritz W, Kelm JM (2013) Multi-cell type human liver microtissues for hepatotoxicity testing. Arch Toxicol 87:209–213
32. Boos JA, Misun PM, Michlmayr A, Hierlemann A, Frey O (2019) Microfluidic multitissue platform for advanced embryotoxicity testing in vitro. Adv Sci (Weinh) 6:1900294
33. Lee S-A, No DY, Kang E, Ju J, Kim D-S, Lee S-H (2013) Spheroid-based three-dimensional liver-on-a-chip to investigate hepatocyte–hepatic stellate cell interactions and flow effects. Lab Chip 13:3529–3537
34. Bhise NS, Manoharan V, Massa S et al (2016) A liver-on-a-chip platform with bioprinted hepatic spheroids. Biofabrication 8:014101
35. Waldbaur A, Rapp H, Länge K, Rapp BE (2011) Let there be chip—towards rapid prototyping of microfluidic devices: one-step manufacturing processes. Anal Methods 3:2681–2716
36. Vernetti LA, Senutovitch N, Boltz R et al (2016) A human liver microphysiology platform for investigating physiology, drug safety, and disease models. Exp Biol Med (Maywood) 241:101–114
37. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS (2004) Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell 6:483–495

38. Ma C, Zhao L, Zhou E-M, Xu J, Shen S, Wang J (2016) On-Chip construction of liver lobule-like microtissue and its application for adverse drug reaction assay. Anal Chem 88:1719–1727
39. Banaeiyan AA, Theobald J, Paukštyte J, Wölfl S, Adiels CB, Goksör M (2017) Design and fabrication of a scalable liver-lobule-on-a-chip microphysiological platform. Biofabrication 9: 015014
40. Ho CT, Lin RZ, Chen RJ et al (2013) Liver-cell patterning lab chip: mimicking the morphology of liver lobule tissue. Lab Chip 13:3578–3587
41. Kang YBA, Eo J, Mert S, Yarmush ML, Usta OB (2018) Metabolic patterning on a chip: towards in vitro liver zonation of primary rat and human hepatocytes. Sci Rep 8:8951
42. Du Y, Li N, Long M (2018) Liver sinusoid on a chip. Methods Cell Biol 146:105–134
43. Lee PJ, Hung PJ, Lee LP (2007) An artificial liver sinusoid with a microfluidic endothelial-like barrier for primary hepatocyte culture. Biotechnol Bioeng 97:1340–1346
44. Rennert K, Steinborn S, Gröger M et al (2015) A microfluidically perfused three dimensional human liver model. Biomaterials 71:119–131
45. Weng YS, Chang SF, Shih MC, Tseng SH, Lai CH (2017) Scaffold-free liver-on-a-chip with multiscale organotypic cultures. Adv Mater 29
46. Domansky K, Inman W, Serdy J, Dash A, Lim MH, Griffith LG (2010) Perfused multiwell plate for 3D liver tissue engineering. Lab Chip 10:51–58
47. Ye N, Qin J, Shi W, Liu X, Lin B (2007) Cell-based high content screening using an integrated microfluidic device. Lab Chip 7:1696–1704
48. Li L, Gokduman K, Gokaltun A, Yarmush ML, Usta OB (2019) A microfluidic 3D hepatocyte chip for hepatotoxicity testing of nanoparticles. Nanomedicine (Lond) 14:2209–2226
49. Deng J, Chen Z, Zhang X et al (2019) A liver-chip-based alcoholic liver disease model featuring multi-non-parenchymal cells. Biomed Microdevices 21:57
50. Gori M, Simonelli MC, Giannitelli SM, Businaro L, Trombetta M, Rainer A (2016) Investigating nonalcoholic fatty liver disease in a liver-on-a-chip microfluidic device. PLoS One 11:e0159729
51. Kim J, Lee C, Kim I et al (2020) Three-dimensional human liver-chip emulating premetastatic niche formation by breast cancer-derived extracellular vesicles. ACS Nano 14:14971–14988
52. Bovard D, Sandoz A, Luettich K et al (2018) A lung/liver-on-a-chip platform for acute and chronic toxicity studies. Lab Chip 18:3814–3829
53. Choe A, Ha SK, Choi I, Choi N, Sung JH (2017) Microfluidic gut-liver chip for reproducing the first pass metabolism. Biomed Microdevices 19:4
54. Lee DW, Ha SK, Choi I, Sung JH (2017) 3D gut-liver chip with a PK model for prediction of first-pass metabolism. Biomed Microdevices 19:100
55. Zhang C, Zhao Z, Abdul Rahim NA, van Noort D, Yu H (2009) Towards a human-on-chip: culturing multiple cell types on a chip with compartmentalized microenvironments. Lab Chip 9: 3185–3192
56. Baert Y, Ruetschle I, Cools W et al (2020) A multi-organ-chip co-culture of liver and testis equivalents: a first step toward a systemic male reprotoxicity model. Hum Reprod 35:1029–1044

Placenta on Chip: A Modern Approach to Probe Feto-Maternal Interface

16

Anshul Bhide, Afrah Aboo, Mamta Sawant, Abhijit Majumder, Debjani Paul, and Deepak Modi

Abstract

The placenta is the primary link between the mother and the fetus. It regulates the exchange of nutrients, oxygen, and waste between the mother and the fetus. It also protects the fetus from maternal insults such as infections and exposure to toxicants, thereby minimizing any teratogenicity. Therefore, ensuring placental health is essential for a successful pregnancy. However, the development, differentiation, functions, and dysfunctions of the human placenta are difficult to study in vivo due to ethical constraints. On the other hand, the differences in the morphologies and the functions of the placenta between different species make the data obtained from animal models less reliable. Availability of various human placental cell lines as well as recent advances in microfluidics and bioprinting technologies has led to the evolution of the placenta-on-chip models. These models can effectively simulate the anatomical and physiological characteristics of the human placenta by successfully mimicking the in vivo environment inside a microfluidic chip. By incorporating relevant placental cell types into the device design and controlling the microenvironment, microfluidics-based in vitro models hold clues to the pathogenesis of placental dysfunction and facilitate drug testing across the maternal-fetal interface. In this chapter, we highlight some existing microfluidic platforms that have attempted to simulate various

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aspects of placental biology. In addition, we review the applications of these models and discuss the limitations in the context of placental physiology and pathophysiology.

16.1 Introduction

Disorders of pregnancy due to placental defects are almost unique to the human species. The placenta-related pregnancy disorders include miscarriage, gestational diabetes, preterm births, and pre-eclampsia. Accounting for more than a third of human pregnancies, these disorders are a cause of significant morbidity and mortality both to the mother and the fetus. Thus, an understanding of the placental functions in normal conditions and in pathological pregnancies is crucial for developing management strategies for these disorders. Pregnant women and hence their fetuses are treated with a significant number of prescription and non-prescription medications. As the mother undergoes significant adaptations during pregnancy, the pharmacokinetics and pharmacodynamics of drugs can change, resulting in changes in effectiveness of the therapies. Unfortunately, pregnant women are excluded from most drug studies as there are concerns regarding fetal risk and hence the data on placental-mediated drug interactions are sparse. Thus, studying the placenta and investigating placental dysfunctions are of interest to clinicians, developmental and reproductive biologists, and toxicologists. Therefore, versatile in vitro models that simulate human placenta both anatomically and physiologically are highly desirable.

Advancements in the availability of human placental cell lines, microfluidics, and bioprinting have contributed to the evolution of placenta-on-chip models. Over the conventional two-dimensional (2D) systems, the three-dimensional (3D) cell culture systems, the placenta-on-chip models, are multicellular in nature recapitulating the spatial organization observed in vivo, reiterating some aspect of the tissue functions. The microfluidic systems provide mechanical cues like shear stress, tension and compression which has an important role in cell/tissue physiology. These bioengineered in vitro models of human placenta promise to offer precise control over the microenvironment allowing recapitulation of specific aspects of human placentation in health and disease.

Herein, we discuss (1) development and the structure of human placenta; (2) current placenta-on-chip models; (3) their applications and limitations in studying placental physiology and disorders of pregnancy; and (4) present limitations and critical aspects for future considerations.

16.2 Development and Structure of Human Placenta

Fertilization in humans is characterized by fusion of sperm and egg and formation of a zygote. This zygote then undergoes multiple rounds of cell division and forms a blastocyst. The blastocyst stage comprises Inner Cell Mass (ICM), Trophectoderm

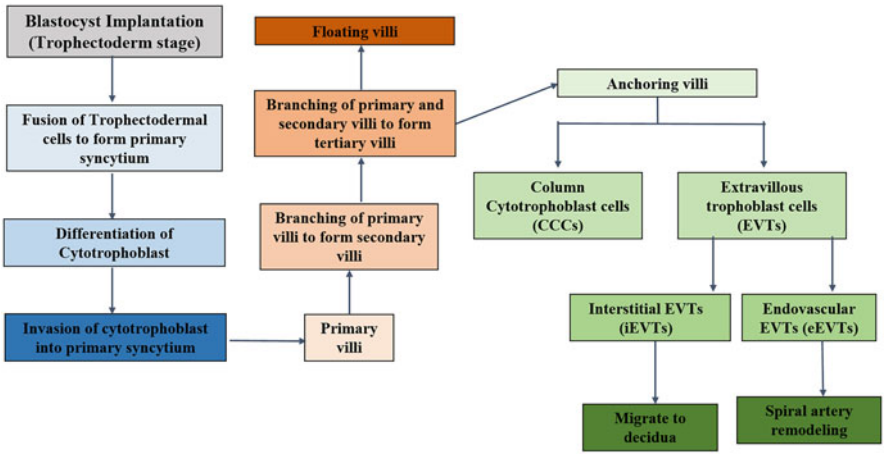

Fig. 16.1 Schematic process of formation of the Human Placenta

(TE), and the blastocoel. The inner cell mass develops into the embryo proper, while the trophectoderm cells give rise to the placenta. Before the formation of placenta, the blastocyst implants itself into the maternal uterus. At the time it enters the endometrial lumen, the blastocyst hatches itself from the zona coat and is closely apposed to the wall of the endometrium termed as the luminal epithelium. This is followed by the attachment stage where the contact between trophectoderm and the luminal epithelium of the uterus is established. The next step is embryo invasion wherein the trophectoderm invades into the endometrium to successfully embed the embryo into the uterus. The trophectoderm invasion marks the beginning of placenta development [1]. The sequelae of events involved in formation of human placenta are summarized in Fig. 16.1 and described below. The reader is requested to go through the following papers for a detailed description of placental development [2–5].

1. **Primary syncytium formation**: The trophectoderm after initial invasion gets completely embedded inside the endometrium. By this stage, the embryo is bathed by endometrial secretions. The trophectoderm cells proliferate and expand in numbers and differentiate into cytotrophoblasts. The cytotrophoblasts, by a fusion event, lose their cell boundaries and form multinucleated syncytiotrophoblast surrounding the embryo forming the primary syncytium. This primary syncytium further invades and erodes the decidua.
2. **Formation of primary villi**: While the outer syncytial layer is in direct contact with the maternal blood, the inner layer of the cytotrophoblast cells completely surrounds the conceptus to form the cytotrophoblast shell. At this stage, the cytotrophoblast proliferates and solid outgrowths of cytotrophoblast cells protrude into the syncytiotrophoblast layer giving rise to the primary villi. In a cross

section, the primary villus has a central core surrounded by the layer of syncytiotrophoblasts.

3. **Development of the secondary and tertiary villi**: Towards the embryonic side, the extraembryonic mesoderm is differentiated and layers above the cytotrophoblast cells. The cells of the extraembryonic mesoderm extend and penetrate into the primary villi, resulting in the inner stromal core, the cytotrophoblast layer, and the outer syncytiotrophoblast layer. The fetal blood vessels that originate from the extraembryonic mesoderm then penetrate into the primary villus making them vascular. By the process of constant proliferation and invasion, the primary villi branches into secondary villi and tertiary villi resulting in the placental tree. The endothelium in the villi never penetrates into the trophoblast lacunae or anastomose with the material blood vessels. Numerous "daughter" villi arise out of the tertiary villi. These remain either free and project into the intervillous space.
4. **The floating villi**: The villi that bathe in maternal blood are referred to as the floating villi, and through the syncytiotrophoblast layer, there is exchange of nutrients and exclusion of waste products between the mother and fetus. These floating villi act as a selectively permeable structure and are primarily responsible for the barrier function of the placenta. The gases, nutrients, and waste products that diffuse through the maternal and fetal blood must pass through a total of four layers. From outside to inside, these are (1) Syncytiotrophoblast, (2) Cytotrophoblast, (3) Loose connective tissue that surrounds the endothelium, (4) Capillary endothelium of the villus. These four elements together form the placental barrier of the first trimester which continues to be so till 16 weeks (fourth month of human pregnancy). After 16 weeks, cytotrophoblast in the tertiary villi disappears and the villi branches further and becomes very thin. This results in reduction of the intervillous space and the fetal vessels become smaller and come in direct contact with the outermost syncytiotrophoblast layer. By the end of the second trimester, the barrier is now a bilayered structure with the outer syncytiotrophoblast layer and the fetal endothelium. After the mid second trimester, the gases, nutrients, and waste products that diffuse through the maternal and fetal blood must pass through these two layers to enter the fetal circulation.
5. **Anchoring villi**: These are villi that attach to the maternal decidua and are determinant of the depth of placentation. The major cell type of the anchoring villi are the extravillous trophoblasts. The cytotrophoblast shell at the proximal end of the growing villi facing the maternal decidua proliferates and forms a column of cells termed as the cytotrophoblast cell column. These column trophoblasts anchor on to the maternal decidua and initially plug the maternal arteries (Fig. 16.2). The cells at the tip of column trophoblast cells that anchor on the decidua leave the cytotrophoblast column and gain an invasive phenotype to form the extravillous trophoblast (EVTs) cells. These EVTs further differentiate into interstitial EVTs (iEVTs) and the endovascular EVTs (eEVTs). The iEVTs reside in the endometrial bed, while the eEVTs remodel the spiral arteries.

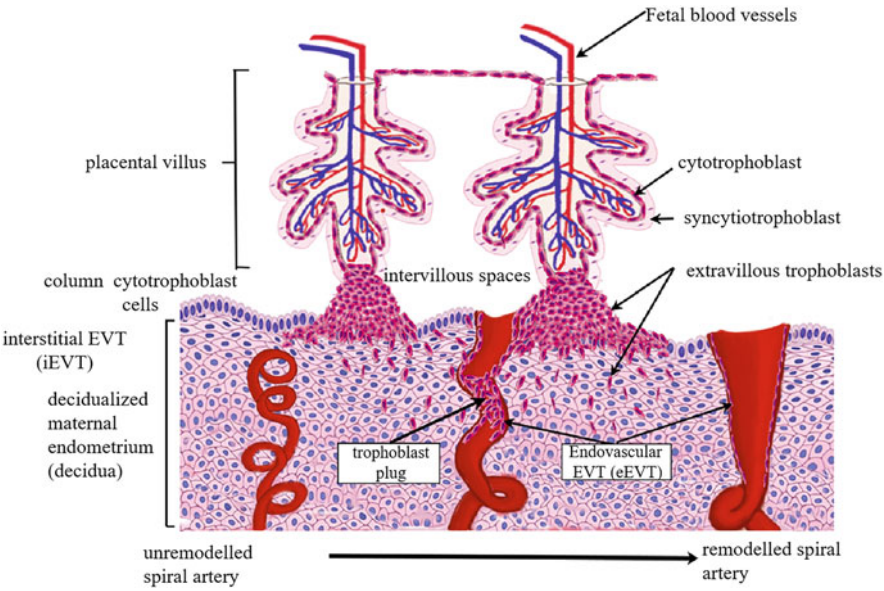

Fig. 16.2 Anchoring villus, column trophoblast, extravillus trophoblasts and the process of spiral artery remodeling. At the tip of the anchoring villi, the syncytiotrophoblast anchor and form the cell columns. These cells are known as cytotrophoblast column cells. These cytotrophoblast cells initially plug the lumen of the spiral arteries and differentiate to form the Extravillous trophoblasts (EVTs). The EVTs are of two types, interstitial EVTS (iEVT) and endovascualr EVTs (eEVTs). The invasive EVTs invade both into the maternal decidua and the lumen of the spiral arteries in the first trimester of pregnancy. The eEVTs remodel the spiral arteries by replacing endothelial lining to accommodate a high flow volume of maternal blood without excessive velocity. Not all the arteries undergo the process of remodeling some remain in natural state as well. (Figure adapted with permission from Saghian et al. © (2019) The Royal Society (UK))

6. **Spiral artery remodelling**: Spiral artery remodelling is the process where the endothelial cells of the artery are replaced by the eEVTs to give rise to a vessel that is capable of high conductance under physiological pressure. The eEVTs of the column trophoblasts invade the maternal arteries and displace the maternal endothelium and the surrounding smooth muscle cells. This occurs well under the upper two thirds of the decidua basalis almost until the myometrium.

16.3 Structure Function Relationship of the Human Placenta in Physiology and Pathophysiology

In this section, we will describe the functions ascribed to the main cell types in the placenta and their roles in pregnancy.

- **Cytotrophoblasts and syncytiotrophoblasts**: The placental villi are made of two cell populations, viz., the undifferentiated cytotrophoblasts and fully differentiated syncytiotrophoblasts.
- **Syncytiotrophoblast cells**: This is the outermost layer of all villi and covers all placental villi as one single layer. Beneath the syncytiotrophoblasts are the cytotrophoblasts which are the precursors of syncytiotrophoblasts. Cytotrophoblasts continually differentiate into syncytiotrophoblasts during villous formation and development. The syncytiotrophoblast fetal cells are directly in contact with maternal blood and the cell layer acts as a primary barrier between the mother and fetus and is the site with the highest metabolic and endocrine activity. This layer plays a key function in the materno-fetal (and feto-maternal) transfer of biomolecules, drugs, and infectious agents. It is an active site for catabolism and synthesis of proteins and lipids. It is also a site of hormone synthesis of β-hCG (beta-subunit of human chorionic gonadotropin) and progesterone which are essential to maintain pregnancy [6]. The syncytiotrophoblast is the main site of exchange for drugs and a determinant of mother to child transmission of pathogens. In general, hydrophobic low molecular weight xenobiotics (<600 Da) can usually transfer across the placenta via passive diffusion, while hydrophilic and/or high molecular weight compounds (1000+ Da) cross very poorly [7]. The syncytiotrophoblast layer also protects the fetuses from maternal infections. However, some viruses (like Zika, SARS-CoV-2, CMV, EBV) can infect the syncytiotrophoblasts and act as reservoirs of the viruses [8–11]. In some instances, the viruses can also undergo lytic cycles in the placental cells and release the virions into the feto-maternal infection causing mother to child transmission. Beyond viruses, pathogens like malarial parasites have specific affinity to the placenta as they can reside into the syncytiotrophoblasts causing placental malaria which has life-threatening consequences [12, 13].
- **Extravillous trophoblasts**: The extravillous trophoblasts are of two types, viz., the endovascular EVTs (eEVTs) and the interstitial EVTs (iEVTs). The extravillous trophoblast cells that infiltrate arterial walls of the spiral arteries make up the eEVTs. The EVTs that migrate into the maternal decidua are iEVTs. The migration of EVTs is a crucial step in human placentation. During pregnancy, the maternal blood vessels are extensively remodelled up to the upper third of the myometrium. To achieve this, the cells of the column trophoblasts migrate into the decidua. These iCTBs surround the maternal spiral arteries and initiate the remodelling process. Once the iCTBs align the maternal spiral arteries, they differentiate into eCTBs by adopting a vascular adhesion and interdigitate into the endothelial layer, and eventually replace the maternal endothelial cells completely. This allows the narrow spiral with relatively high resistance to be transformed into highly dilated, low-resistance channels (Fig. 16.2). These remodelled vessels alter the blood flow dynamics at an appropriate speed to ensure maximal perfusion and prevent damage to the villi.

  Along with spiral remodelling, the eCTBs also form trophoblast plugs in early pregnancy to obstruct spiral arteries and prevent blood flow. As a consequence,

the placenta exists in a low oxygen environment for the majority of the first trimester, and this is thought to be key to promote placental development, vasculogenesis, and angiogenesis. The failure of deep endovascular invasion and spiral artery remodelling is observed in a number of pregnancy-related disorders. Premature loosening of the trophoblast plugs is associated with miscarriages; inadequate spiral artery remodelling is observed in pregnancies complicated with pre-eclampsia and Intrauterine Growth Restriction (IUGR) [14].

16.4 Placenta-on-Chip

Since placental functions are crucial for several aspects of pregnancy, research in this direction has taken a centre stage. Attempts have been made to simulate various aspects of placental physiology using in vitro model systems. In the following sections, we have reviewed the existing models of human placenta-on-chip. Table 16.1 lists the cell lines derived from human placenta that are used for developing the placenta-on-chip models.

The existing placenta-on-a-chip models can be classified into those that simulate the placental barrier function and those that are used to study trophoblast migration. The placental barrier models can be sub-classified into two design categories: multi-layered vertical design and single-layered planar design (Fig. 16.3).

In the multi-layered vertical design, two microfluidic layers containing the microchannel features are aligned and bonded with an extracellular matrix membrane between them, mimicking the placental barrier that separates the maternal circulation in the intervillous space and fetal circulation in the fetal capillaries (Fig. 16.4). In these models, the cells are first introduced into the lower channel and the device is immediately inverted to allow cells to adhere to the bottom surface of the membrane. During this process, the inlets and outlets are blocked to prevent loss of cell suspension by outflow. After the cell adherence is confirmed, cells are

Table 16.1 Cell lines of human placenta used for developing the placenta on Chip models

Cell lines	Origin	Characteristic
BeWo	Choriocarcinoma	Cytotrophoblast
JEG3	Choriocarcinoma	Extravillous trophoblast
HTR8/SVneo	Primary cell lines	Extravillous trophoblast
Human umbilical vein endothelial cells (HUVEC)	Primary cell lines	Endothelial cells
Human placental vascular endothelial cells (HPVECs)	Primary cell lines	Endothelial cells
Human villous mesenchymal fibroblasts (HVMF)	Primary cell line fibroblasts	Fibroblasts cells

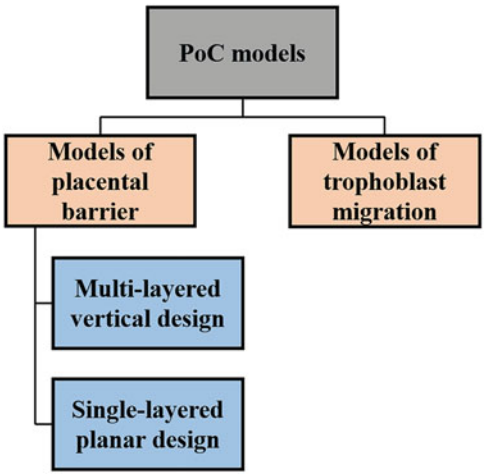

Fig. 16.3 Classification of the placenta-on-chip (PoC) models

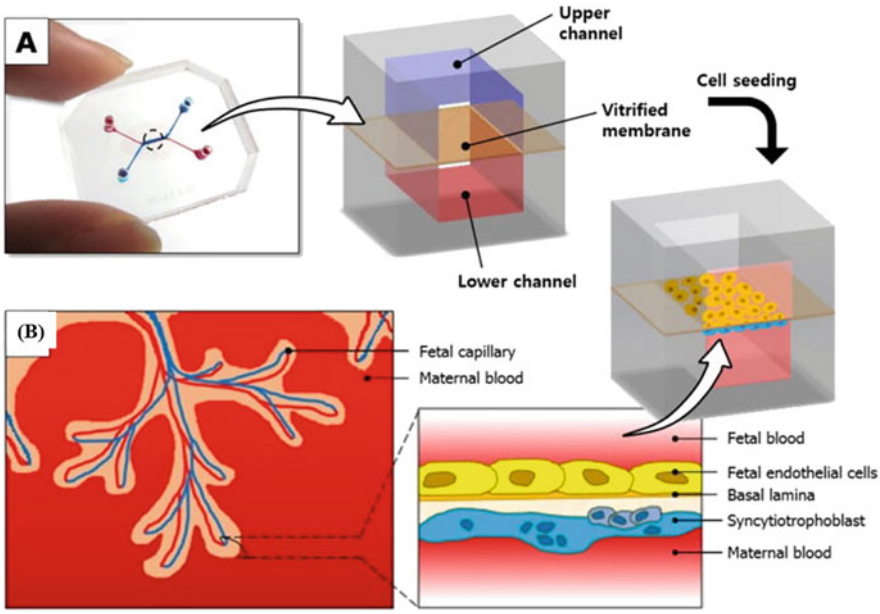

Fig. 16.4 Vertical multi-layered placenta-on-chip model. (**a**) The engineered device is composed of two PDMS chambers separated by a vitrified collagen membrane. The upper surface is shown in blue and the lower surface is marked in red. (**b**) Trophoblasts are co-cultured with endothelial cells in close apposition on the either side of the membrane to form a microengineered placenta-on-a-chip device which mimics the placental barrier function. (The figure is adapted with permission from Lee et al. © (2016) Journal of maternal-fetal and neonatal medicine)

introduced into the upper microchannel and allowed to adhere. Once cells are seeded and firmly attached to the membrane surfaces, they are allowed to form confluent monolayers, while media is flowed through the channels at physiologically relevant flow rates.

In the single-layered planar design, a single microfluidic layer is patterned to define both the maternal and fetal channels in the same layer and bonded to an unpatterned surface. The maternal and fetal cells are seeded on either side of a separating membrane or matrix and cultured under media perfusion at physiologically relevant flow rates.

16.4.1 Models of Placental Barrier

The first-ever placental barrier model was developed by Lee et al. [15], containing two patterned polydimethylsiloxane (PDMS) layers with a vitrified collagen membrane sandwiched between them (Fig. 16.5). The PDMS layers, with channel features of width 500 μm and height 200 μm, were fabricated using soft lithography techniques. The vitrified collagen membrane was developed using type 1 collagen mixed with distilled water and culture medium. This solution was dispensed over the central region of the lower PDMS microchannel and allowed to gelate, which was

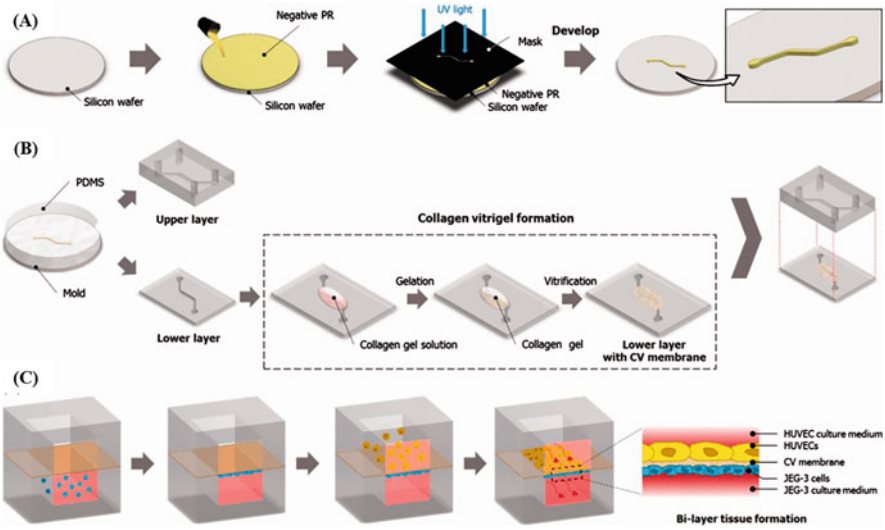

Fig. 16.5 Fabrication of the vertical multi-layered placenta-on-a-chip: (**a**) The microchannel master is produced using photolithography. (**b**) Replica molding of PDMS is carried out to define microchannels. The vitrified collagen membrane is incorporated to define the placental barrier. The upper and the lower PDMS slabs are permanently bonded, housing a microchannel covered with the vitrified membrane. (**c**) Sequential seeding of JEG-3 cells on the maternal side and HUVECs on the fetal side in the multilayered device. (The figure is adapted with permission from Lee et al. © (2016) Journal of maternal-fetal and neonatal medicine)

then dried overnight to develop into the membrane. The two PDMS layers, with the membrane attached to the lower layer, were plasma treated for bonding to each other. The membrane surface on the fetal and maternal sides were coated with fibronectin and gelatin, respectively, prior to cell seeding to enhance cell adhesion and growth. JEG-3 human trophoblast cells and human umbilical vein endothelial cells (HUVECs) were seeded on the maternal and fetal side of the vitrified collagen membrane, respectively. After cells were firmly attached to the membrane, appropriate growth media were perfused through the channels using a syringe pump at 30 μL/h for 3 days to allow the cells to form confluent monolayers. This model recapitulated the structural aspect of the placental villous tree of the feto-maternal interface (Fig. 16.4). This device was tested for functionality by comparing the efficiency of glucose transport across the barrier on-chip with ex-vivo models. The rate of transfer and the permeability coefficient of glucose were found similar to that of ex-vivo model systems. However, this design used Jeg-3 cells which resemble the EVTs and not the syncytiotrophoblasts. Thus, the results from this study need to be interpreted with caution.

The above design from Lee et al. 2015 was extended by Blundell et al. who used a semipermeable polycarbonate membrane with 1 μm pores between the maternal and fetal channels [16, 17]. The two PDMS layers containing the microchannels ($w = 1$ mm, $h = 135$ μm, $l = 1.5$ cm) were bonded to the membrane using adhesive PDMS mortar. The channels were treated with fibronectin before seeding the cells. BeWo human trophoblasts and human primary placental villous endothelial cells (HPVECs) were cultured on either side of the membrane with culture media flowing through the channels at 100 μL/h. The device was developed to study the transport of substances from mother to fetus under continuous physiological flow conditions. This model was tested to study the transfer of glyburide (a drug used for treatment of gestational diabetes) across the placental barrier. They were able to show that glyburide transport across the barrier was very low and this finding was similar to that of other ex-vivo models (see below).

Another attempt was made to mimic the placental barrier structure in vitro. Mosavati et al. developed a model with BeWo trophoblast cells and HUVECs cultured on either side of a type 1 collagen-coated polycarbonate membrane with 0.4 μm sized pores [18]. The PDMS layers, with channel features of width 1 mm and height 200 μm, were bonded to the membrane using adhesive PDMS/toluene mortar. The cells were cultured in the microchannels under static conditions for 3 days with media change every day. Cell confluency was achieved at 72 h post cell seeding for HUVEC (86 ± 3%) and BeWo cell (90 ± 2%) layers. Intercellular junction formation for both the cell types was assessed by staining the cells using calcein-stain for HUVEC cells and Cell Tracker orange fluorescence-stain for BeWo cells growing on each surface of the porous membrane. Effects of membrane porosity and flow rate on glucose diffusion across the placental barrier were studied using numerical models. Glucose transfer rates of the device were determined in the absence of cells (93 ± 5.7%), in the presence of single cell type (70 ± 6.1% for HUVECs and 63 ± 4.2% for BeWo cells), and co-culture (35 ± 2.5%) which were similar to their glucose transfer rates determined by numerical models. Different

flow rates (10–150 μL/h) were also assessed to see the effect of variable flow rate on transport of glucose across the chamber. Their results showed that at 50 μL/h, glucose concentration in the top channel is 6.81 mM and 5.76 mM in the bottom channel. At 150 μL/h, diffusion was negligible with which they concluded that the rate of glucose diffusion decreases with the flow rate which was concordant to their numerical model data. Effect of membrane porosity (0.01 and 0.1) was also checked on glucose transfer. The difference in the glucose concentration between the maternal and the fetal chamber was greater in the 0.01 porosity membrane as compared to the 0.1 porosity membrane. This occurred due to the lower resistance to mass transfer from the higher membrane porosity, suggesting that glucose diffusion increases with increasing membrane porosity.

16.4.2 Modifications of the Barrier Design

1. *Single-layered design*: Miura et al. developed a similar placental barrier model with trophoblast cells [19]. However, in their design they used BeWo cells that mimic the syncytiotrophoblasts which are key cells involved in barrier function. No cells were seeded in the fetal channel making it a single-layered device. The device consisted of a 15 mm maternal and a 20 mm fetal channel, both of 2 mm width and 200 μm height - patterned on two PDMS layers by soft lithography. The device has a multi-layered vertical design in terms of the microfluidic layers, but has only one type of cell, making it single-layered in terms of the cellular layers in the device. A 4 mm circular chamber for cell seeding was made with a biopsy punch in the maternal channel and sealed using a thin PDMS membrane. BeWo cells were adhered to a 10 μm thick vitrified collagen membrane and they studied how fluid shear stress (FSS) affects the formation of microvilli under static and dynamic flow conditions. In the static condition, cells had sparse microvillar surfaces. Under flow conditions, the entire cell surface in the maternal chamber was covered with microvilli. The FSS was low at the centre of the chamber ($\sim$0.001 dyn/cm^2) as compared to the site near inlet and outlet of the microvillar ($\sim$0.1 dyn/cm^2). At the centre of the chamber, microvillar protrusions were long ($>$2 μm); however, near the site of inlet and outlet, the microvillar protrusions were shortened ($<$2 μm). The microvilli formation induced by FSS was quantified, by measuring total length of microvilli/field from the scanning electron microscopy (SEM) images for each FSS condition. The measured lengths of microvilli were significantly different under high- and low-FSS conditions. Also, the microvilli formation was increased 10.8-fold at the low-FSS area and 5.6-fold at the high-FSS area compared to the static culture conditions.
2. *Multicellular multi-layered design:* Most of the on-chip platforms use filter membrane as scaffold to act as barrier, but these membranes affect cell behaviour as well as add a level of barrier which is not present in in vivo conditions. Therefore, a different multi-layered model was developed by Kreudar et al. called Membrick, which is a cell culture insert designed to be used in 24 well culture plates (Fig. 16.6) [20]. The model is a 3D bioprinted gelatin methacrylate

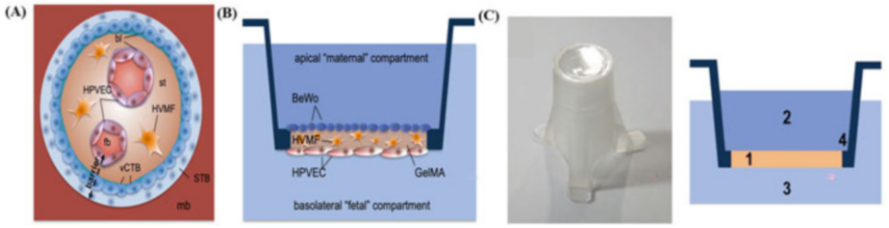

Fig. 16.6 Schematic of placental cross-section (First trimester) and biological membrane system (Membrick) (**a**) placental villus contains fetal blood vessels (fb), endothelial cells (HPVEC), basal lamina (bl), mesenchymal stroma (st), human villous mesenchymal fibroblasts (HVMF), villous cytotrophoblast cells (vCTB), syncytiotrophoblast (STB) maternal blood (mb); (**b**) Scheme of the placental barrier model ofthe Membrick, the biological membrane is made with methacrylated gelatine (GelMA) with human villous mesenchymal fibroblasts (HVMF) embedded in it. BeWo and Human placental vascular endothelial cells (HPVEC) are cultured on the apical and basal side on the membrane respectively; (**c**) Depiction of cell culture insert scheme; biological membrane (1) two compartments for cell seeding (2, 3). The Membrick device can be kept hanging in 24 well by the device arms; the bottom of the cylindrical body includes a pipette rest for facilitated media exchange (4). (Figure is adapted from Kreudar et al. 2020)

(GelMA) biomembrane, which was used as an alternative to filter membranes. The model replicated early-stage placental barrier with human villous mesenchymal fibroblasts (resembling the stromal tissue of placental villi) embedded in a 3D bioprinted gelatin methacrylate (GelMA) biomembrane. The bioink was polymerised by light initiated polymerization using a 3D bioprinter to form the hydrogel membrane with a thickness of 330–400 μm within the Membrick body. After 3 days of culture with fibroblasts, the basolateral side of the membrane was seeded with HPVECs, while the apical side was seeded with BeWo trophoblast cells and cultured for 12 days with media change every day. Electrical resistance was measured to assess biomembrane integrity. The membrick showed lower resistance 18.22 ± 1.6 cm^2 as compared to polyethylene terephthalate (PET) membrane 36.2 ± 2.8 cm^2 devices. Membrick permeability was tested by using different weight molecules [457 Da Lucifer Yellow (LY), 3 kDa Dextran-Texas Red (DTR) and 70 kDa Dextran-FITC (DF)] in serum-free medium. After 24 h, permeation of smaller weight molecules (457 Da LY and 3 kDa DTR) was observed similar in both PET (10.37 μg/mL/cm^2 LY and 10.1 μg/mL/cm^2 DTR) and biomembrane (10.83 μg/mL/cm^2 LY and 10.13 μg/mL/cm^2 DTR). For 70 kDa DF PET membrane displayed a low hindrance (8.62 μg/mL/cm^2) after 24 h, but the biomembrane showed much reduced biological membrane (2.41 μg/mL/cm^2). This trilayer design was tested for successful growth of each type with cell-specific markers (HPVEC-CD31; BeWo-cytokeratin 7; HVMF-Vimentin) and showed a proof-of-concept study of culturing 3 cell types on a device.

3. *Design to simulate the barrier functions with villi-like projections:* During the course of development, the placenta undergoes several structural changes which affect the surface area, cellular arrangement, and size of the organ. Thus, the barrier design described above was modified to resemble the in vivo like placental

villous structure [21]. Using 2 photon polymerization which allows precision in structural patterns like external shape, pore size, and internal porosity of fabricated structures, Mandt et al. developed a planar placental barrier model wherein a separating GelMOD-AEMA hydrogel membrane of 100 μm thickness was fabricated within a microfluidic chip. The chip was fabricated in poly-(ethylene glycol)-dimethacrylate (PEGdma) and consisted of an X-shaped chamber with an intersection area of width 1 mm and length 1.4 mm. The GelMOD-AEMA membrane was fabricated at the intersection region, separating the chamber into a maternal and fetal compartment, and coated with fibronectin. The membrane consisted of five consecutive loops to mimic the villous structure of the placental barrier. BeWo trophoblast cells and HUVECs were seeded on the maternal and fetal sides of the membrane. After cells were attached, appropriate growth media were perfused through the channels at 50–70 μL/h. This design was made to assess the barrier function of the placenta. The barrier integrity was checked using riboflavin and fluorescently labelled dextran. Riboflavin was observed to pass through the barrier, while dextran did not. This design provides a much closer platform to study the barrier function as the hydrogel barrier and the villi-like projections would be similar to in vivo conditions compared to a membrane.

16.4.3 Applications of the Placenta-on-Chip Devices that Simulate the Barrier Function

1. *Maternal infection: E. coli* is the predominant microorganism observed during infections and was chosen as a model to simulate these conditions. The presence of an immune response against the bacteria and whether it can cross the barrier was tested using the placenta-on-chip model. The design was principally similar to that described above with minor modifications. Zhu et al. cultured BeWo human trophoblast cells and HUVECs on the maternal and fetal channel sides of a semipermeable polycarbonate membrane [22]. The membrane was attached to the upper PDMS layer by electrostatic interaction and the bottom surface of the membrane was smeared with PDMS glue. Following this, the glue was cured and the two PDMS layers were plasma bonded together. The channels ($w = 1.5$ mm, $h = 400$ μm, $l = 1.5$ cm) were coated with type 1 collagen to promote cell adherence and growth before seeding the cells. Growth media were perfused through the channels at 10 μL/h using a syringe pump, resembling the maternal and fetal circulation in vivo. To simulate maternal infection, *E. coli* was applied to the maternal side, and the cells were tested for inflammatory responses. An increased secretion of inflammatory cytokines by trophoblasts and the adhesion of maternal macrophages was observed in response to *E. coli*. There was significant cell death in both the compartments, mimicking cell death during membrane rupture. Due to bacterial infection, there was a surge of inflammatory cytokines in both the chambers. The integrity of the fetal cell monolayer was also compromised, i.e. the endothelial barrier was disrupted. Overall, this model was

shown to simulate not just the barrier functions, but also that of membrane rupture in women experiencing preterm delivery.

2. *Drug transport from mother to child:* Caffeine is a widely consumed xenobiotic and studies have shown its transfer from the maternal to the fetal chamber which could cause long-term damage to the developing fetus as it lacks the enzymes which could inactivate caffeine. Therefore, it is essential to understand the rate of caffeine transfer across the placenta. Naltrexone is a medication prescribed for opioid use disorder with unknown potential harm to the fetus. To address if placenta-on-chip devices are useful in studying drug transport, Pemathilaka et al. utilized the placental barrier model [23, 24]. Essentially a design similar to that described above, two layers of PDMS, with channel features of width 400 μm and height 100 μm, were bonded by plasma treatment with the membrane sandwiched between them. The membrane was coated with an E-C-L (Entactin–collagen IV–laminin) solution and BeWo trophoblast cells and HUVECs were cultured on either side of a polyester track etched membrane with 0.4 μm sized pores under media perfusion at 50 μL/h. The integrity of the device and cells cultured was tested using cell type-specific antibodies. They observed a steady transfer of caffeine from the maternal to the fetal chamber. In this device, the transport of 6β-naltrexol across the placental barrier was also studied and the authors found that ~ 2.5% of the total maternal concentration of 6β-naltrexol could be passed on in the fetal chamber. The authors also demonstrated that the HUVECs in the fetal chamber had altered expression of cytokines, suggestive of the harmful effect of the compound on the fetal cells. Thus, this study provides proof of concept on application of this device in drug transport from mother to child.
3. *Nanoparticles:* Nanomedicine is gaining momentum and there are several studies demonstrating its therapeutic usefulness. The risk of exposure to such particles in pregnant women in vivo is yet unknown. These exposures can lead to production of reactive oxygen species. Reactive oxygen species are unstable, free radical oxygen carrying molecules which are highly reactive. Increase in ROS can cause damage to DNA, RNA, and protein which could lead to cell death. Two studies have reported the effect of nanoparticle exposure on the placental function (mainly the placenta's barrier function). Schuller et al. developed a multi-layered placental barrier model consisting of a porous PET membrane of pore size 3 μm and thickness 9 μm coated with type 1 collagen with an interdigitated electrode (impedance microsensor) array for nanoparticle risk assessment [25]. The channel features were patterned on microscope glass slides of height 1 mm and the layers were bonded together using double-sided adhesive tape. BeWo trophoblast cells were adhered to the membrane and the placental barrier integrity with exposure to nanoparticles was monitored. This model was tested using different concentrations of nanomaterials made of silicon dioxide (SiO_2), titanium dioxide (TiO_2), and zinc oxide (ZnO), which are present in various cosmetics and day to day consumables and further assessed with Reactive oxygen species (ROS) production. To show that a confluent layer of BeWo cell line was achieved on the device, tight junction immunofluorescence protein Zonula occludens protein 1 (ZO1), which was seen to be intact at all the cell junctions. Molecule transport

across the microsensor membrane showed that their procedure did not affect the cellular function. A comparative study of the newly designed interdigitated membrane-bound impedance sensor array model vs standard nanotoxicological viability assays including Presto Blue and reactive oxygen species (ROS) assays was done. TiO_2 and SiO_2 devices showed no loss of barrier integrity after 24 h of exposure which was found in line with Presto blue viability assay. Reactive oxygen species were found to be produced equally in both models after exposure with both types of nanoparticles. ZnO nanoparticles in both standard and on-chip models showed similar levels of increase in ROS after 4 h and 24 h of exposure. The increasing ROS in turn created a similar level of cytotoxicities after 4 h and 24 h of exposure, respectively. Yin et al. developed a single-layered planar microfluidic device consisting of a PDMS layer with a central matrix channel and two parallel channels on either side of it [26]. This PDMS layer was bonded to an unpatterned PDMS slab after plasma treatment. Matrigel was perfused into the central channel of width 300 μm and height 50 μm and allowed to gelate. BeWo and HUVECs were introduced into the side channels and cultured on either side of the matrigel extracellular matrix by tilting the device after seeding. After 24 h of culture, appropriate growth media flowed through the channels at 20 μL/h. On the device, they were able to show that increasing concentration of nanoparticles would directly affect the viability of the cells in both maternal and fetal chambers which implied that it could cause placental barrier to rupture. Increase of inflammatory molecules in both the chambers indicates severe effects of these nanoparticles on the placental function. This model system has a high potential for assessment of various drug delivery systems and biomedical research purposes.

16.4.4 Placenta-on-Chip Models of Trophoblasts Migration

In models of trophoblast migration, trophoblast invasion or migration in response to chemical gradients is studied. Unlike the barrier function devices, there are very few designs available to study invasion. Most of the studies are limited to an early proof of concept and very few applications have been tested.

16.4.4.1 Devices to Study Trophoblast Migration in Response to Chemical Gradients

Abbas et al. developed a trophoblast invasion model using a PDMS device with a central channel and two side channels, each of length 2 cm, width 1.3 mm, and height 150 μm, separated by micropillars (Fig. 16.7) [27]. The PDMS layer was fabricated by soft lithography techniques and plasma bonded to a glass coverslip. Matrigel embedded with primary extravillous trophoblasts was loaded into the central microchannel and allowed to polymerise. One of the side channels was perfused with media containing granulocyte-macrophage colony-stimulating factor (GM-CSF), while the other side channel was perfused with media alone (control), both at 50 μL/h for 12 h. The model was used to study the migration of the

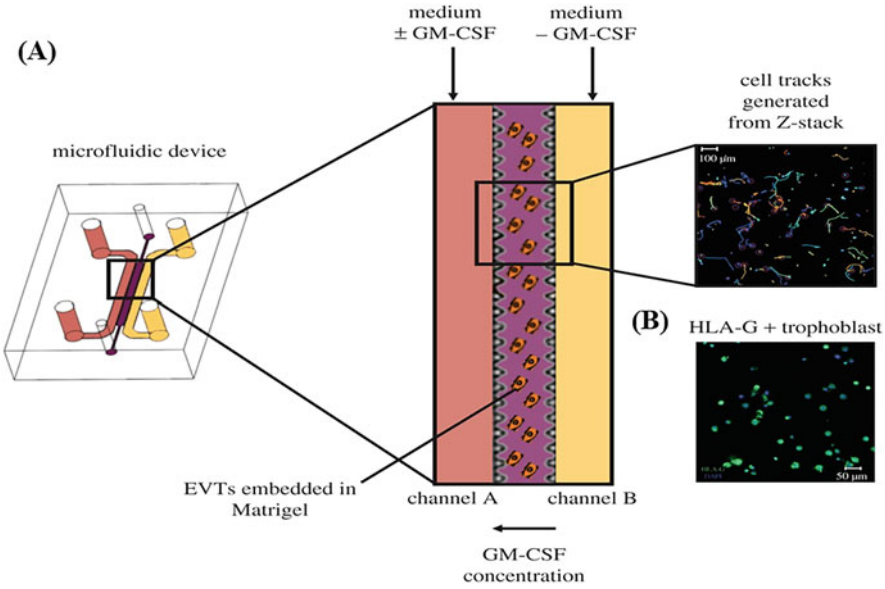

Fig. 16.7 Placenta-on-chip model for studying trophoblast invasion. (**a**) The microfluidic device consists of a central channel and two side channels (channel A and B). EVTs isolated from first trimester placenta are stained with a cell tracker and are embedded in a Matrigel matrix in the central channel. Media with and without GM-CSF are perfused through channel A and channel B, respectively, creating a gradient of GM-GSF across the central channel. The migration of the cells in the central channel is tracked using time lapse microscopy. (**b**) The purity of the cells embedded in the central channel was confirmed by immunostaining of EVT for HLA-G (green) and staining the nucleus of each cell using DAPI dye. (Figure is adapted with permission from Abbas et al © (2017) the Royal Society)

trophoblasts under the influence of the cytokine GM-CSF. The migration of the trophoblast cells in the absence of GM-CSF was unidirectional, whereas in the presence of GM-CSF, cellular migration was increased with increasing directionality and velocity. This approach allows accurate estimation of migration of individual cell types' response to chemo attractants by quantifying their migration speed, directionality, and pattern of migration (single/collective).

While the design described above finds application in studying trophoblast migration, the application is restricted to chemical gradients and is a monocellular design. To circumvent this limitation, Yong Pu et al. developed a 3D microfluidic platform to mimic the trophoblast microenvironment for studying trophoblast invasion in vitro (Fig. 16.8) [28]. The platform, fabricated in PDMS, consisted of a central compartment and two outer channels ($w = 200$ µm, $h = 100$ µm) on either side of it, separated by a barrier of width 50 µm consisting of pillars. After coating the channels with fibronectin, HUVECs and extravillous trophoblasts (HTR8/SVneo cells) were seeded in the central compartment and the outer channels, respectively. Growth media were perfused through the channels at 0.6 µL/h using syringe pumps.

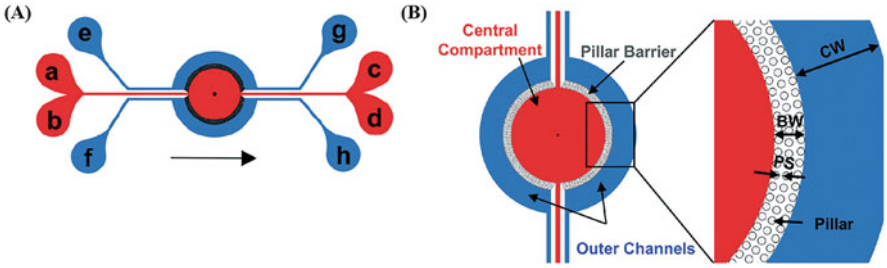

Fig. 16.8 3D planar multilayer chip (**a**) 3D microfluidic chip scheme depicting the following components: (1) a central compartment (red) with a central feeder line supplied by two inlet ports (a and b) connected to two outlet ports (c and d); (2) two outer channels (blue) with two outer feeder lines supplied by two inlet ports (e and f) connected to two outlet ports (g and h), and a (3) pillar barrier in between. Arrow corresponds to the directionality of medium flow; (**b**) Close up scheme of the center of the 3D microfluidic chip with the central compartment (red), outer channels (blue, width (CW): 200 μm), and pillar barrier (width (BW): 50 μm), filled with pillars (pillar spacing (PS): 3 μm). (Figure adapted permission from Pu et al. © (2021) Royal society of chemistry)

The model was used to study the invasion properties of the trophoblast cells. To achieve a confluent monolayer of cells on the device, three matrices, Gelatin, Matrigel, and Fibronectin, were tested. Attachment of cells was found on all the three matrices, but fibronectin had the highest cell attachment maximum. Integrity of the barrier and barrier permeability was assessed using FITC-Dextran diffusion. The cell layers on the pillar and device restricted the diffusion of FITC-dextran into the central compartment and resulted in a gradual build-up gradient reaching the centre of the central compartment by 72 h. They assessed the invasion of HTR8/SVneo cells under the influence of folic acid which aids in the invasion of the extravillous trophoblast cells in vivo. They observed enhanced trophoblast migration from the outer chamber (HTR8/SVneo) to the central compartment (HUVECs) in the device.

16.4.4.2 Design to Simulate Spiral Artery

Kuo et al. developed a 3D printed bioengineered placenta model using gelatin methacrylate (GelMA) to mimic the features of maternal decidua for studying trophoblast migration/invasion (Fig. 16.9) [29]. The cylindrical model was loaded with different components such as cells, ECM, and growth factors at different radial positions. BeWo trophoblast cells were embedded in the GelMA hydrogel at the periphery of the construct, while epithelial growth factor (EGF) was loaded at the centre. EGF gradients were created to understand migration patterns. This bioengineered model is designed to recapitulate spiral artery remodelling under dynamic flow conditions. The use of this model is to study the migration of cells under the influence of a stimulus. Using this model, they showed a positive correlation between migration pattern of trophoblast cells and epidermal growth factor (EGF). This design can be used to understand pharmacological and molecular interactions of therapeutic agents for pre-eclampsia.

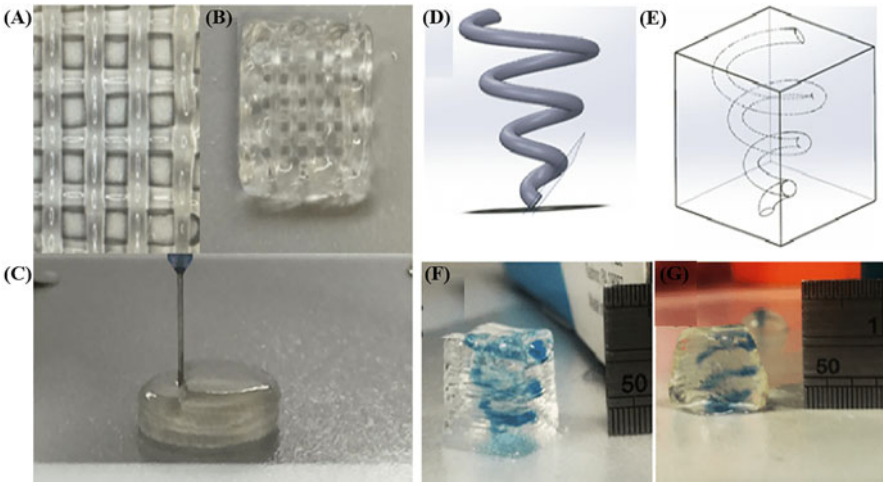

Fig. 16.9 Characterization of the GelMA printing platform (**a** and **b**) Lattice structures with 1 × 1 mm spacing. (**c**) Solid disc structure (diameter = 10 mm). (**d**) CAD model for spiral (height, 10 mm; channel diameter, 1 mm; pitch, 3 mm; taper helix angle, 20°; 3 revolutions). (**e**) Overall CAD model used for 3D printing: spiral embedded in a cube. (**f**) 3D printed spiral (blue) in a cube (transparent): side view. (**g**) 3D printed spiral (blue) in a cube (transparent): cross-sectional view. (Figure adapted with permission from Kuo et al. © (2016) American Chemical Society)

16.5 Limitation of the Placenta-on-Chip Devices

There are multiple reported designs of placenta-on-chip which have attempted to simulate the barrier functions or trophoblast migration. However, most of these are still at the proof of concept stage and very little advancement is made in terms of design improvements or applications. Listed below are some major limitations that the placenta-on-chip devices pose. It is important that one is aware of these limitations while interpreting the data from various studies. Most of the placenta-on-chip designs have a single layer of trophoblast cells or they are coupled with HUVECs. Such bilayer devices, mimic the floating villus, are similar to those of term placenta villi. The first and second trimester placenta villi are three to four layered structures (see above) and the molecules need to pass through these layers to reach the fetus. This aspect is not captured in the existing devices. The floating villus consist of cytotrophoblast cells and syncytiotrophoblast cells, each having different gene expression patterns as well as variable affinities towards biomolecules for their transport from maternal to fetal chamber. BeWo cells have cytotrophoblast-like properties and they need to be synctialized to become syncytiotrophoblast cells, making this device less homologous to the in vivo conditions. However, most models have used cytotrophoblast cells as the representative of the trophoblast layer, which is not physiologically relevant. The physiological shear stress levels

at the feto-maternal interface are less explored, but studies have tried to calculate levels of shear stress in vivo [30–32]. The shear stress levels experienced in vivo are difficult to recapitulate in these devices. Also, the placental tissue produces a lot of hormones like βhCG, progesterone, oestrogen, etc. All the models have failed to test this important feature of the placental tissue.

The process of migration in vivo is assisted by multiple factors produced by various cell types. All the model devices used to study the migration pattern have used only one such factor to show mere tendency of highly migratory cells to react towards stimulus. Migration of trophoblast cells is influenced by other cell types in vivo. For example, decidual cells stimulate the migration and allow passage of these trophoblast cells by secreting various pro-invasive factors [33, 34]. None of these devices have taken the true physiology into consideration for performing these studies.

16.6 Future Direction

In the field of placenta-on-chip (PoC), the recent research progress gives hope of using these devices for toxicity testing, drug testing, and in understanding the basic physiology of the placenta in the near future. However, like any other organ-on-chip models, PoCs are also facing some serious challenges as described below. The community needs to address these issues together.

First, every PoC is to be validated for the application for which it is fabricated. For example, if a PoC is developed as the model for transplacental barrier, they should be validated for the same. However, there is sufficient ambiguity in terms of how a PoC should be validated. Due to the lack of any broadly accepted standard, it is presently not possible to comment if a PoC model is mimicking the in vivo conditions reliably. We also need to keep in mind that a PoC model validated for one molecule may not give reliable results for a different molecule of very different size, charge, and diffusivity. However, developing separate models for separate systems and validating them individually every time against some standard is next to impossible. Hence, the community should come together to set standards against which a newly developed PoC model can be validated.

To resolve this issue, engineering knowledge merged with an understanding of placental biology can be very useful. In any transport process such as transport of biochemicals across the placental barrier, surface to volume ratio is important. It becomes further critical when we reach micron scale level as in microfluidic devices and tissue capillaries. Other important factors are flow velocity, thickness of the boundary layers, transport parameters such as diffusivity and viscosity, porosity of the membrane and the extracellular matrix, etc. The researchers need to estimate the range of these parameters in the human placenta and then to formulate one or more relevant dimensionless numbers that can be matched between in vivo and in vitro when designing a new device. Further, unlike many other organs, the placenta is a continuously developing dynamic structure. The development of the placenta happens over a specific timescale. While designing PoC models which are generally static, one needs to also consider the dynamic behaviour of the placenta.

Like most of the organ-on-chip devices, PoCs are also mostly made of PDMS, which is known to absorb hydrophobic molecules. Additionally, while PDMS is an excellent polymer for lab scale demonstration of a concept, it is not suitable for large scale production. Hence, to make PoCs popular among researchers, the researchers should search for alternative materials such as polymethyl methacrylate (PMMA) or polystyrene (PS). Another major issue with PoCs is availability of relevant cells. The cell type to be used in PoC models should be physiologically relevant. Quite often, the cell type used in PoC devices are carcinoma cells which do not truly represent a healthy placenta. The other alternative is to use primary cells isolated from aborted placenta. However, the availability of such primary cells is limited, and they often have serious ethical and sociocultural concerns. A plausible alternative is the creation of placenta-specific immortalized cell lines made from the tissues of healthy and consenting adult donors.

Last but not the least, microfluidic experiments are often not very easy to set up by the end user. To maintain the flow precisely in a microfluidic device, many accessories such as pumps, valves, and tubings are needed. Such additional accessories make the device bulky and difficult to handle. A slightly faulty operation may lead to situations such as backflow and leakage. Hence, if such devices are not designed to be of "Plug and Play" type, the researchers trained in biology may find a barrier in adopting the technology for regular use. Hence, it is important to keep the design and operation of the microfluidic platform simple. Ideally, the device should be easy to use, modular, and robust. Collection of samples and imaging of the cells should also be hassle-free in the device. However, doing so should not compromise the critical features of an in vivo placenta that the device intends to mimic. A very simple model may be excellent for analysis and imaging, but it may miss many important structural and functional features of placenta. On the other hand, a complex model may be able to mimic various aspects of the placenta, but may offer a significant challenge in operation. Striking the balance is a challenge for a PoC designer.

16.7 Conclusion

This chapter has presented the recent developments in designing and application of PoC devices. We have discussed various available models, their advantages, and limitations. With the development of placenta-specific cell lines, modular designs, and integration of the knowledge of biology and engineering, PoC devices can be invaluable models for drug and toxicity testing in near future.

References

1. Ashary N, Tiwari A, Modi D (2018) Embryo implantation: war in times of love. Endocrinology 159:1188–1198. https://doi.org/10.1210/EN.2017-03082
2. Turco MY, Moffett A (2019) Development of the human placenta. Development 146. https://doi.org/10.1242/DEV.163428/223131

3. Aplin JD, Ruane PT (2017) Embryo–epithelium interactions during implantation at a glance. J Cell Sci 130:15–22. https://doi.org/10.1242/JCS.175943
4. Burton GJ, Jauniaux E (2018) Development of the human placenta and fetal heart: Synergic or independent? Front Physiol 9:373. https://doi.org/10.3389/FPHYS.2018.00373/BIBTEX
5. Burton GJ, Redman CW, Roberts JM, Moffett A (2019) Pre-eclampsia: pathophysiology and clinical implications. BMJ 366. https://doi.org/10.1136/BMJ.L2381
6. Yabe S, Alexenko AP, Amita M et al (2016) Comparison of syncytiotrophoblast generated from human embryonic stem cells and from term placentas. Proc Natl Acad Sci U S A 113:E2598–E2607. https://doi.org/10.1073/PNAS.1601630113/-/DCSUPPLEMENTAL
7. Rubinchik-Stern M, Eyal S (2012) Drug interactions at the human placenta: What is the evidence? Front Pharmacol 3:126. https://doi.org/10.3389/FPHAR.2012.00126/BIBTEX
8. Ashary N, Bhide A, Chakraborty P et al (2020) Single-cell RNA-seq identifies cell subsets in human placenta that highly expresses factors driving pathogenesis of SARS-CoV-2. Front Cell Dev Biol 8:783. https://doi.org/10.3389/FCELL.2020.00783/BIBTEX
9. Shende P, Gaikwad P, Gandhewar M et al (2021) Persistence of SARS-CoV-2 in the first trimester placenta leading to transplacental transmission and fetal demise from an asymptomatic mother. Hum Reprod 36:899–906. https://doi.org/10.1093/HUMREP/DEAA367
10. Adibi JJ, Marques ETA, Cartus A, Beigi RH (2016) Teratogenic effects of the Zika virus and the role of the placenta. Lancet 387:1587–1590. https://doi.org/10.1016/S0140-6736(16)00650-4
11. Ostrander B, Bale JF (2019) Congenital and perinatal infections. Handb Clin Neurol 162:133–153. https://doi.org/10.1016/B978-0-444-64029-1.00006-0
12. Mbachu II, Ejikunle SD, Anolue F et al (2020) Relationship between placenta malaria and mother to child transmission of HIV infection in pregnant women in South East Nigeria. Malar J 19:1–8. https://doi.org/10.1186/S12936-020-03171-2/TABLES/6
13. Ayisi JG, Van Eijk AM, Ter Kuile FO et al (2003) The effect of dual infection with HIV and malaria on pregnancy outcome in western Kenya. AIDS 17:585–594. https://doi.org/10.1097/01.aids.0000042977.95433.37
14. McNally R, Alqudah A, Obradovic D, McClements L (2017) Elucidating the pathogenesis of pre-eclampsia using in vitro models of spiral uterine artery remodelling. Curr Hypertens Rep 19:1–13. https://doi.org/10.1007/S11906-017-0786-2/TABLES/2
15. Lee JS, Romero R, Han YM et al (2016) Placenta-on-A-chip: A novel platform to study the biology of the human placenta. J Matern Neonatal Med 29:1046–1054. https://doi.org/10.3109/14767058.2015.1038518
16. Blundell C, Tess ER, Schanzer ASR et al (2016) A microphysiological model of the human placental barrier. Lab Chip 16:3065–3073. https://doi.org/10.1039/c6lc00259e
17. Blundell C, Yi YS, Ma L et al (2018) Placental drug transport-on-a-chip: a microengineered in vitro model of transporter-mediated drug efflux in the human placental barrier. Adv Healthc Mater 7:1–17. https://doi.org/10.1002/adhm.201700786
18. Mosavati B, Oleinikov AV, Du E (2020) Development of an organ-on-a-chip-device for study of placental pathologies. Int J Mol Sci 21:1–12. https://doi.org/10.3390/ijms21228755
19. Miura S, Sato K, Kato-Negishi M et al (2015) Fluid shear triggers microvilli formation via mechanosensitive activation of TRPV6. Nat Commun 6:1–11. https://doi.org/10.1038/ncomms9871
20. Kreuder AE, Bolaños-Rosales A, Palmer C et al (2020) Inspired by the human placenta: a novel 3D bioprinted membrane system to create barrier models. Sci Rep 10:1–14. https://doi.org/10.1038/s41598-020-72559-6
21. Mandt D, Gruber P, Markovic M et al (2018) Fabrication of biomimetic placental barrier structures within a microfluidic device utilizing two-photon polymerization. Int J Bioprinting 4:1–12. https://doi.org/10.18063/IJB.v4i2.144
22. Zhu Y, Yin F, Wang H et al (2018) Placental barrier-on-a-chip: modeling placental inflammatory responses to bacterial infection. ACS Biomater Sci Eng 4:3356–3363. https://doi.org/10.1021/acsbiomaterials.8b00653

23. Pemathilaka RL, Caplin JD, Aykar SS et al (2019) Placenta-on-a-chip: in vitro study of caffeine transport across placental barrier using liquid chromatography mass spectrometry. Glob Challenges 3:1800112. https://doi.org/10.1002/gch2.201800112
24. Pemathilaka RL, Reynolds DE, Hashemi NN (2020) Maternally administered naltrexone and its major active metabolite 6β-naltrexol transport across the placental barrier in vitro. bioRxiv 1–31. https://doi.org/10.1101/2020.04.16.045229
25. Schuller P, Rothbauer M, Kratz SRA et al (2020) A lab-on-a-chip system with an embedded porous membrane-based impedance biosensor array for nanoparticle risk assessment on placental Bewo trophoblast cells. Sens Actuators B Chem 312. https://doi.org/10.1016/j.snb.2020.127946
26. Yin F, Zhu Y, Zhang M et al (2019) A 3D human placenta-on-a-chip model to probe nanoparticle exposure at the placental barrier. Toxicol In Vitro 54:105–113. https://doi.org/10.1016/j.tiv.2018.08.014
27. Abbas Y, Oefner CM, Polacheck WJ et al (2017) A microfluidics assay to study invasion of human placental trophoblast cells. J R Soc Interface 14. https://doi.org/10.1098/rsif.2017.0131
28. Pu Y, Gingrich J, Veiga-Lopez A (2021) A 3-dimensional microfluidic platform for modeling human extravillous trophoblast invasion and toxicological screening. Lab Chip 21:546–557. https://doi.org/10.1039/d0lc01013h
29. Kuo CY, Eranki A, Placone JK et al (2016) Development of a 3D printed, bioengineered placenta model to evaluate the role of trophoblast migration in preeclampsia. ACS Biomater Sci Eng 2:1817–1826. https://doi.org/10.1021/acsbiomaterials.6b00031
30. Tun WM, Yap CH, Saw SN et al (2019) Differences in placental capillary shear stress in fetal growth restriction may affect endothelial cell function and vascular network formation. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-019-46151-6
31. Lecarpentier E, Bhatt M, Bertin GI et al (2016) Computational fluid dynamic simulations of maternal circulation: wall shear stress in the human placenta and its biological implications. PLoS One 11:1–18. https://doi.org/10.1371/journal.pone.0147262
32. Sprague B, Chesler NC, Magness RR (2010) Shear stress regulation of nitric oxide production in uterine and placental artery endothelial cells: experimental studies and hemodynamic models of shear stresses on endothelial cells. Int J Dev Biol 54:331–339. https://doi.org/10.1387/ijdb.082832bs
33. Sharma S, Godbole G, Modi D (2016) Decidual control of trophoblast invasion. Am J Reprod Immunol 75:341–350. https://doi.org/10.1111/AJI.12466
34. Godbole G, Suman P, Malik A et al (2017) Decrease in expression of HOXA10 in the decidua after embryo implantation promotes trophoblast invasion. Endocrinology 158:2618–2633. https://doi.org/10.1210/EN.2017-00032

Microfluidic Retina-on-Chip

17

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Abstract

Retina, being a visceral layer of eye, has attracted the attention of researchers all over the world. Due to its complex cellular architecture and coordination, in vitro replication of retinal functions has been a daunting task. Ex-vivo/in vivo animal retinal models are limited by their ethical concerns, cost, reproducibility, and prolonged experimental duration. Over the years, microfluidic perfusion devices have captured significant interest as in vitro models for investigating cellular functions, in a controlled system. Mimicking of retinal architecture and cellular functions via in vitro retina-on-chip (RoC) model has opened newer avenues for understanding retinal-complexity, retinal diseases, and also for high-throughput evaluation of retinal drugs. Development of retinal organoids within RoCs has thus offered potential of reducing the burden on animal investigations, while enabling numerous experimental runs within a relatively short period of time. This chapter emphasizes on the technological advancements in the area of RoC, the fabrication methods employed in device construction, and its application to mimic in vivo retinal milieu for pre-clinical research.

Keywords

Retina-on-chip · Microfluidics · Pre-clinical studies · In vitro models · Organ-on-chip

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17.1 Introduction

In recent years, the organoid technology has revolutionized the entire concept of disease modelling, providing enhanced perspectives about disease progression, and hence, bridging the gap between animal and human trials [1]. Over the past few decades, technologies have evolved from 2-D to 3-D models, the latter including spheroids, organoids, organ-on-chip (OoC), and in vitro humanoid models, which have opened up unprecedented avenues in biological and drug discovery research [2]. The use of embryonic and adult stem cells for developing disease models has created newer opportunities for novel drug screening, development of personalized medicine, bio-banking, lab-on-chip research, and regenerative applications [3]. Successful in vitro models have been created for recapitulating the brain [4], pancreas [5], lungs [6], liver [7], kidneys [8], and mimicking the in vivo anatomy and physiology of these organs. Extensive efforts and research in in vitro retinal models have resulted in prototype tissue constructs bearing huge anatomical and physiological resemblance to the human retina and with the potential to conserve vision [9, 10].

Recent advancements have encouraged the use of alternatives to animal models for ocular investigations. Limitations of animal models, due to prolonged experimental time, cost intensiveness, ethical concerns, lack of reproducibility, and variability in results, stress the need for alternate ex-vivo/in vitro disease models. The ex-vivo organotypic ocular models constructed using isolated rabbit eye (IRE), isolated chicken eye (ICE), and bovine corneal opacity and permeability (BCOP), etc. resemble the human corneal thickness and structure and hence were extensively employed for detecting ocular irritants. But due to existing interspecies variations in anatomical and physiological features of the eye, such as lack of cones sensitivity to red light in rabbits [11], absence of macula, greater photoreceptor cell density in mouse central retina [12], extrapolation of results obtained via ex-vivo animal models to human outcomes becomes challenging [13].

As a result, there was an increasing emergence of in vitro ocular models that were more robust and reproducible. In vitro ocular models were employed for drug development, toxicology, permeability, and formulation-related studies, besides basic and translational research. Due to limitations faced in culturing the primary ocular cells which could be cultivated for only up to ~4–5 passages, their use for in vitro disease model development was challenging. Therefore, in vitro ocular organoid models were developed using immortalized cell lines [14]. Such models require continuous culturing of cells, with growth medium and nutrients being replenished at regular intervals. However, continuous cell culture may lead to altered cellular characteristics and chromosomal modifications [15].

Further, to study blood-retinal barrier, toxicity, gene delivery, and polarity, in vitro retinal co-culture models were developed using retinal pigment epithelium (RPE) and retinal capillary endothelium. These models were developed from primary, immortalized cell lines and other animal (RPE-J) as well as human (ARPE-19) cell lines. Retinal organoids (RO) resembling the in vivo environment, and displaying formation of optic vesicles in the cell culture dish [16, 17], have offered

great potential for disease modelling, drug screening, and validation of therapeutics. However, with static RO, replication of the micro-physiological perfusion system and vascularization is limited [18].

The retina-on-chip (RoC) technology has brought about revolutionary changes in artificial organ construction by allowing disease modelling within a dynamic perfusion system, which mimics in vivo tissue micro-physiology and overcomes the limitations of traditional static cultures [19, 20]. So far, the RoC technology has been able to recapitulate the multiple layers of cornea [21] and retina [22, 23], either as discrete layers or as co-culture systems. However, recapitulating the complete complex 3D architecture of the retina using this technology is still challenging and needs to be explored further. In this book chapter, we have described the RoC technology as an alternative in vitro model to the traditional static cultures and animal models, with emphasis on the need and importance of this emerging area in high-throughput pre-clinical research. Further, fabrication, characterization, and validation assays and challenges faced during RoC development have also been elaborately discussed. Further, a discussion has been presented on the case studies of patient-specific retinal diseases that have been recapitulated using the RoC technology, which may ultimately lead to vision improvement and enable evaluation of therapeutics and development of personalised therapies. Finally, the advantages, limitations, and impact of RoC technology on social, industrial, and economic sectors have been highlighted along with the projected repercussions on science and technology.

17.2 Need for RoC Technology

17.2.1 Morphology and Pathophysiology of Retina

The arrangement of retinal layers from anterior to posterior prospective is as follows; choroid, RPE, photoreceptors—rods and cones, external limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, nerve fibre layer, inner limiting membrane, and vitreous humour [24] (Fig. 17.1, created in biorender). The neural components of the retina are stratified and comprise of six types of neural cells, as stated in Table 17.1. The table also describes various disease conditions associated with these cell types.

The outer choroid, which is build up of blood vessels, supplies oxygen and nutrients to the retinal cells. The choroid is followed by a single layer of Retinal Pigment Epithelium (RPE). The main function of RPE is to provide nutrients to the photoreceptors and to remove any waste matter from this layer [28]. The retinal photoreceptors, lying immediately above the RPE, consist of the rod cells, which are responsible for scotopic vision, and the cone cells, that are responsible for photopic vision. These photoreceptor cells are arranged in such a way that their nuclei form a layer, known as the outer nuclear layer, while their remaining cell structures lie towards the RPE [25]. A junctional complex formed between the photoreceptors and

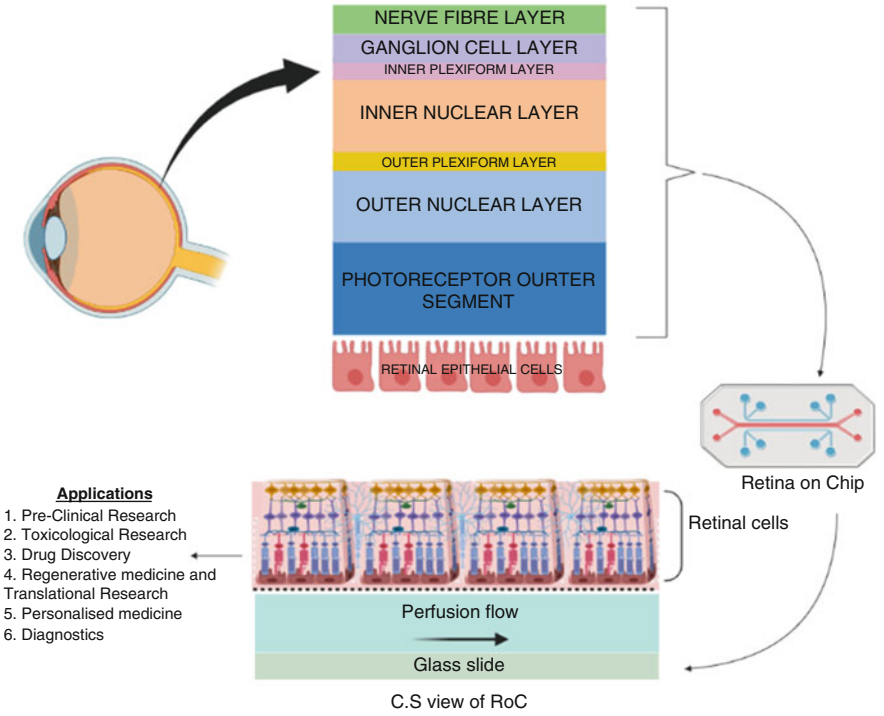

Fig. 17.1 Retinal tissue anatomy and its associated layers that can be mimicked in vitro using RoC technology and its applications

the Müller cells (supporting cells) forms a network-like structural line segment, known as the external limiting membrane or the outer limiting membrane [31].

A segment above the outer nuclear layer, comprising nuclei of the Müllerian glia, the bipolar cells, the amacrine, and the horizontal cells, forms the inner nuclear layer. On either side of the inner nuclear layer lies the outer plexiform layer, which is characterized by association between the photoreceptors, bipolar cells, and horizontal cells, and the inner plexiform layer, which involves association between the bipolar cells, amacrine cells, and retinal ganglion cells [25].

17.2.2 Retinal Diseases

Some common diseases associated with the retina have been depicted in Fig. 17.2. Age-related macular degeneration (AMD) is a condition where the macula (the section of eye that controls sharp vision) becomes thinner by degeneration as the person ages. Currently, there is no treatment for early AMD. However, healthy food intake, injecting anti-VEGF drugs (Vascular Endothelial Growth Factor), and laser surgery are some of the therapies practised to treat this disease [32]. Pre-clinical

Table 17.1 Retinal neural cells, their function, and the disease associated with them

Neural retinal cells	Function	Associated diseases
Photoreceptors (rods and cones)	Capturing and processing of input light [25]	Progressive cone dystrophies (CODs) and cone-rod dystrophies (CORDs) [26] Retinal Degeneration [27]
Horizontal cells	Connecting, integrating, and regulating the input signals from multiple photoreceptor cells and transmitting to the surrounding bipolar cells [28]	Horizontal cells are more prone to become malignant post RB1 gene loss-of-function. Therefore, contributing to metastatic tumours as cell-of-origin, as seen in case of retinoblastoma [29]
Bipolar cells	Interconnecting and conveying signals from photoreceptor cells to retinal ganglion cells [28]	Paraneoplastic retinopathies [29]
Amacrine cells	Integrating and regulating the input signals from bipolar cells to retinal ganglion cells [28]	Tay-Sachs disease [30]
Ganglion cells	Cells extending from the optic nerve to convey signals to and from the brain [28]	Tay-Sachs disease [30]
Müllerian glia	Organizational backbone of the neural retina that is responsible for the structural and functional stability of retina [25]	–

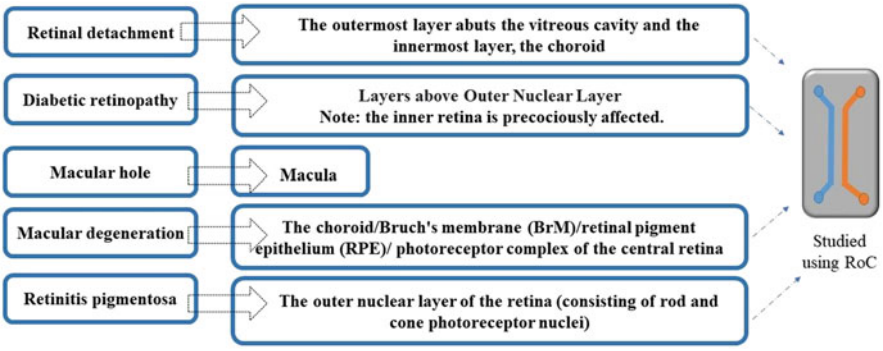

Fig. 17.2 Retinal diseases associated with different retinal layers

evaluation of different anti-VEGF molecules could be conducted in RoCs, reducing the load on animal investigations. When the gel-like content of the vitreous body shrinks, it pulls and causes thinning of the retina, ultimately forming a hole in the retina, which is known as the retinal tear [33]. This condition is currently being treated using laser surgery and cryopexy (freezing technique) [34]. In patients with Tay-Sachs disease, the amacrine cells and ganglion cells of the retina get loaded with

membranous cytoplasmic bodies. This indicates accumulation of ganglioside [30]. Retinal detachment is characterized by detachment of retina from the RPE layer [35]. In diabetic retinopathy, layers above the outer nuclear layer become distorted (reduced) at the superior macular region. However, the photoreceptor-RPE layer is found to be nearly the same as that in the normal eyes [36]. Injecting VEGF inhibitors into the eye, laser surgery (photocoagulation), and vitrectomy are some of the treatments done for patients with diabetic retinopathy [37]. Here, RoC could play a major role in studying the effect of different growth factor inhibitors and fixing the appropriate doses of these. In the case of the macular hole, nerve cells present in the macula are set apart from each other and separate from the eye's surface [38]. Macular holes get healed by themselves and sometimes require vitrectomy to improvise the vision [32].

17.2.3 Evolution of the RoC Technology

Initially, researchers employed 2D models for evaluating drugs related to specific retinal diseases. Primary cultures of RPE cells and retinal capillary endothelial cells, derived from various sources like rats, cows, and even humans, were used by researchers in their investigations to study Diseases (Diabetic Retinopathy, Age-related Macular Degeneration etc.), structure and function of Retina. These monolayer cell culture models were limited by a lower cell viability over longer culture durations. Thus, immortalized RPE cell lines were employed for developing the subsequent models [4]. However, these static 2D cell culture models failed to replicate the physiological interactions between cells and extracellular matrix (ECM) [39]. As a result, 3D cell culture models like retinal spheroids and RO were developed [40].

Subsequently, the static 3D models were advanced into perfusion 3D models within microfluidic devices, the latter offering capability to deal with both spatial and temporal (time) parameters of cellular microenvironments [41]. Various microenvironmental parameters such as oxygen supply, pH maintenance, nutrients perfusion rate, provision of ECM, etc. were optimized in these models to make them fully functional [39, 42]. Therefore, RoCs are in vitro retinal models that use artificial controllable microfluidic systems to culture live retinal cells. These tiny retinal models have the capability to simultaneously analyse the effect of numerous drugs under automated conditions, in a shorter period of time, as compared to the other retinal models [43]. The developments that have been conducted in the area of RoC are depicted in Fig. 17.3 [created in biorender].

There are two types of flows possible within the RoCs, viz. the laminar and the point accession flow. The laminar flow may be used in situations where the spatial control over media flow is not required. However, the use point accession flow has been advocated for controlling the spatial flow of the medium to a specific location within the artificial tissue. Initially, researchers used laminar flow for microfluidic devices, which gradually evolved to point accession flow, due to investigations conducted in brain slices. This was proven by Kirsten and team in their on-retina

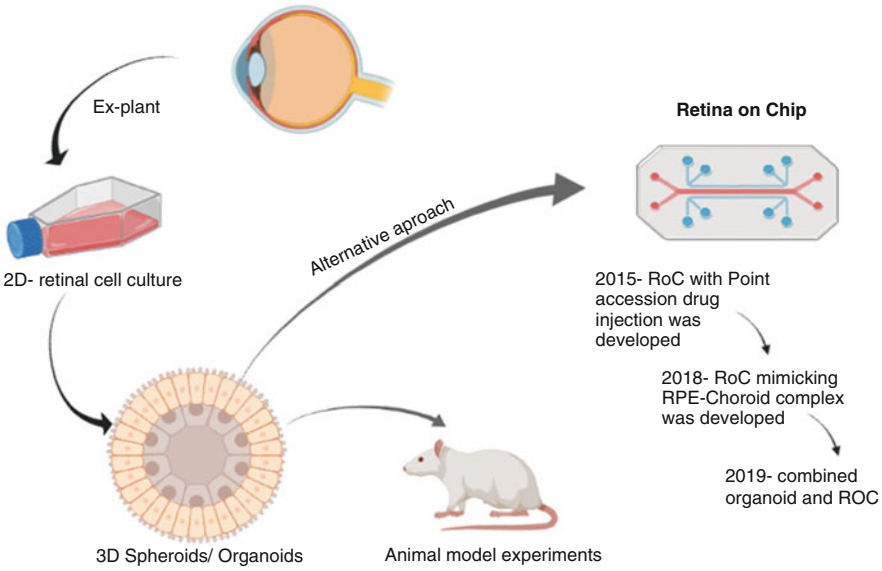

Fig. 17.3 Evolution of RoC, from explant culture to 2D retinal model, animal models, 3D spheroids/organoids and RoC technology, individual and combined

model that employed a point accessible drug flow through RoC. In this study, the entire retina of mice was maintained and cultured within a PDMS device punched with 12 holes of 100 μm diameter. The holes enabled the suction and point accessibility of drug (cholera toxin β), with negative pressure being applied to the bottom of the membrane to hold the curved retina [42].

In 2018, Minchuan and co-workers developed a variant of RoC which mimicked the RPE-choroid complex. The authors suspended HUVECs (Human umbilical vein endothelial cells) and LFs (Lung fibroblasts) in fibrinogen solution, along with thrombin, and loaded them onto the choroid channel of the chip. The gap between choroid channel and RPE channel was filled with fibrinogen and thrombin solution, to mimic the Bruch's membrane. ARPE-19 cell suspension was injected on the apical side of the chip, such that the RPE cells settled and started growing over the gel layer. The final arrangement had a sequential layer of choroid cells (basal side of chip), fibrin gel (mimicking Bruch's membrane), and RPE cell layer (apical of chip). The chip was incubated for 7 days to form the choroid-RPE complex. Development and construction of the outer blood-retinal barrier was the outcome of this chip and it was used to study the effect of bevacizumab on choroidal neovascularization [44].

Further, in 2019, Kevin et al. developed a novel human-derived multilayer tissue model, consisting of both organoids and RoC. The limitations of RO, such as lack of vascularization and inability to provide information regarding interaction between the photoreceptors and RPE, were overcome in this model. The retinal organoids in this model were created using hIPSCs, which had more than seven types of retinal cells that interacted with the RPE through the photoreceptor cells. The chip was

fabricated in such a way that perfusion of medium/drug was possible through the porous PDMS membrane to the organoid-RPE system. This novel RoC was used for evaluating the drug retinopathic side effects of chloroquine and gentamicin [39].

17.3 Applications of RoC

The RoCs that support culturing of retinal cells in an artificial tissue-like system are an unconventional but effective technique to supplement the animal models. It is anticipated to expedite the pre-clinical investigations of drugs meant for retinal disorders. Various diseases like age-related macular degeneration, retinitis pigmentosa, Stargardt disease, etc. lack adequate treatments, mainly due to the unavailability of suitable in vitro models for evaluating developmental drugs [45]. RoCs involving combination of organoids and OoCs have resulted in a complex sequence of ocular layers of interconnected tissues, in the controlled environment of microfluidic chips, to enable both in situ and ex-situ analyses [39].

Researchers like Christopher Probst highlighted the challenges associated with the recapitulation of complex tissue architectures of the human retina solely using engineering approaches. Kevin Achberger and colleagues have proposed a solution based on a combination of stem cell-derived retinal cell complexes (layers of RO in contact with layers of RPE), maintained in a versatile microenvironment, with nutrients/drug delivery systems that mimicked blood vessels [43]. Apart from drug discovery and development, RoCs also open up the possibility of supporting the development of personalized medicines. Therefore, considering the advantages of RoCs over conventional in vitro models as described in Sect. 17.6, various RoC models have been developed in the last decade. In this section, we will discuss some of those models and their applications in basic and clinical research.

17.3.1 Drug Testing

Drug testing plays a major role in the field of toxicology. One of the successes of the target drug lies in fixing a correct dose of the target drug that shows maximum activity and at the same time exhibiting less or no toxicity. Scientists usually use 2D monolayer or suspension cultures of retinal cells as the preliminary test which is followed by testing pre-clinically in laboratory small animals to analyse the toxicity of the target drug towards the retinal diseases. Advantageously, RoC plays a combinational role here as it is an in vitro model mimicking the conditions of the in vivo animal model. For example, ROCs developed by merging organoid and OoC technology have been employed to conduct toxicological and other drug development investigations with anti-malarial drugs like chloroquine and antibiotic drugs like gentamicin. After culturing the cells within the chip, they were treated with chloroquine at different concentrations, for 3 days. Thereafter, cellular viability was estimated by staining propidium iodide (PI) staining and the function of lysosomes to digest the dead cells was analysed by co-staining with lysosomal marker protein

LAMP2. It was observed that 80 mg/mL of chloroquine resulted in strong signals from both PI and LAMP2, which indicated its toxicity to the cells [39]. Similarly, the effect of bevacizumab on inhibiting neo-angiogenesis was studied by Minchuan and team by developing a 3D RPE–choroid complex. The model was able to replicate a blood vessel network complexed with RPE monolayer. Different doses of VEGF (10 ng/mL and 100 ng/mL) were used to induce sprouting of blood vessels. Thereafter, 0.3 mg/mL of bevacizumab was administered to understand its effect on inhibiting the sprouting of blood vessels. It was reported that bevacizumab was able to inhibit networking of blood vessels as well [46]. Therefore, these RoCs as discussed in this chapter are found to be a multifunctioning tool that aids in drug testing, visualising, disease model development, etc. which uplift itself above other cell culture models.

17.3.2 Analysing Functions of Cells/Tissues

Each and every cell or tissue in our body possesses different functions like insulation (fat cells), reproduction (sperm cells and egg cells), protection (epidermal cells, RPE) etc. Once the functions of the specific cells are determined, the need and the importance of that cells in the specific location can be found out. Similarly, the presence of the RPE layer in protecting the cells beneath them from any targeted drug is analysed by Achberger and team using a RoC. The effect of gentamicin on retina using two RoC designs, one with only RO and the other with RO and RPE, was studied. Cells in RoC were treated with different concentrations of gentamicin, namely low concentration (0.5 mg/mL) and high concentration (2.5 mg/mL), for 6 days. In the case of the model based only on RO, an insignificant increase in signal of PI was observed with both the doses. But in case of RoC based on RO and RPE, a significant increase in signal of PI was observed due to treatment of cells with both the doses, indicating that even the lower dose of gentamicin resulted in insignificant toxicity. Hence, it was concluded that RPE provided protection from toxic effects of gentamicin. Therefore, this RoC model helped in understanding the function of cells/tissue in response to treatment with therapeutic molecules [39].

17.3.3 Point Accession Signalling Studies

Drug delivery at a specific point on the target organ is called point accession drug delivery. Microfluidic devices are used to perform this point accession drug delivery. This type of drug delivery has a peculiar application as the investigators are able to study the signalling events that happen via administered drug using point accession reagent delivery. Microfluidic RoC technique with respective accession points is preferred for localized and controlled exposure to drugs. The shape of the retina is one of the hindrances in developing such an on-chip model. Since the retina is a curved organ, researchers had to flatten it using a tissue placement fixture using negative pressure to convert it into a planar structure. Researchers used the flow or

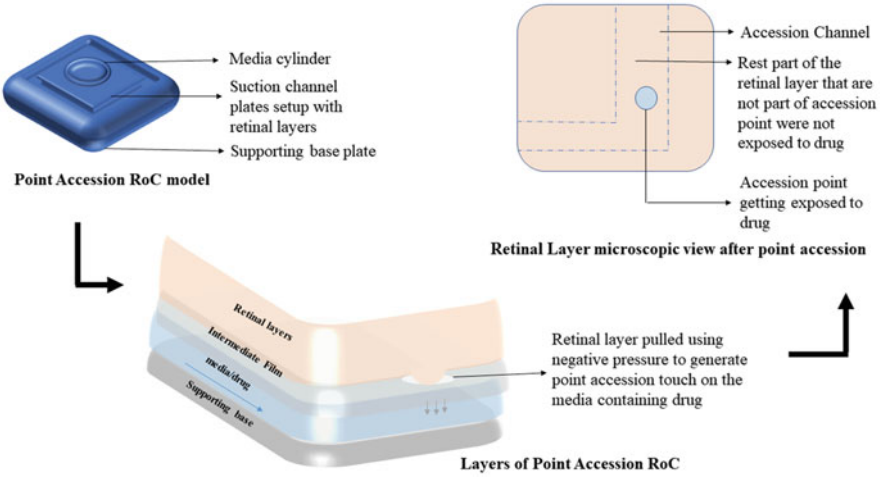

Fig. 17.4 Point accession RoC model using negative pressure to generate point accession touch and its microscopic view

suction caused at the perfusion chamber to flatten the retinal tissue and provide a point-specific exposure of the retinal tissue to the injected drug solution (Fig. 17.4) [42]. Therefore, this model implies that RoC holds this specific application of point accession signalling studies over other retinal models.

17.3.4 Development of Disease Models and Tissue Morphogenesis

Choroidal neovascularization (CNV) is the main cause for macular degeneration. It is a condition where abnormal new blood vessels emerge from the choroid and penetrate into the sub-RPE layer through bruch's membrane. The actual molecular and cellular mechanism that describes the pathogenesis of CNV is not yet clearly understood. The VEGF is well-known to stimulate neo-angiogenesis. Minchuan and team designed a variant of RoC to mimic the RPE-choroid complex. It contained sequential layers of choroid cells (basal side of chip), fibrin gel (mimicking bruch's membrane), and RPE cells (apical of chip). Upon addition of VEGF to the apical side of the chip, new blood vessels from choroid penetrated towards the RPE through the gel, which recreated the CNV disease model. This RoC-CNV model was then used to study the effect of bevacizumab in inhibiting neovascularization of new blood vessels, by studying reduction in sprout length [44].

17.4 Fabrication of Microfluidic RoC Model

Microfabrication and microfluidic techniques are used to develop ROC models. These techniques can be used to replicate micron-sized length scale such as 50–100 μm mimicking amacrine-bipolar cell interactions [47], recapitulate complex co-culture conditions such as across Bruch's membrane [48] blood-retinal barrier [49], and controlled transfer and effect of molecules [47]. Based on operation, there are two types of microfluidic devices, namely, diffusion-based and flow-based [44]. The diffusion-based devices are used for studies where analysis of secreted molecules is important or when shear stress is not needed, etc. The flow-based devices, where pumps are used to maintain the flow of fluid through the device, are widely used for studying the effect of shear stress on cells or removal of secreted molecules in the system. Further, microfluidic devices offer experimental flexibility, better control over culture conditions, automation and reproducibility, and scope for real time analysis, in addition to minimum requirement of cells and reagents [45]. In this section, we will briefly discuss methods used to fabricate the microfluidic devices and studies specific to RoC devices.

17.4.1 Microfabrication Methods to Create RoCs

Various microfluidic RoC designs have been fabricated for developing the retinal tissue under in vitro conditions. Since well-controlled microfluidic devices can be fabricated using lithography, this technique is commonly used to develop microfluidic assays and RoC models. In this method, masks are used to selectively expose photoresist to an energy source, such as light or UV (Fig. 17.5a), and are hence referred as photolithography. Processing of photoresist after exposure to the energy source results in the formation of a desired pattern [50]. Depending upon the warranted resolution, sources such as e-beams, laser, etc. have been used, which have resulted in different variations of this technique, such as photolithography, soft lithography, e-beam lithography, X-ray lithography, etc. In terms of ROC development, lithography-based μ-retina platforms were developed to study migration of retinal progenitor cells (RPCs), in response to chemoattractant stromal-derived factor-1 [51]. Complex cellular functions, such as formation of synaptic connections, can also be studied using microfluidic platforms. For example, Su et al. developed a microfluidic device to study synaptic connections in dissociated retinal precursor cells (R28) [16]. In this model, R28 cells were cultured in compartments separated by microfluidic channels of ~100 μm length, to study synaptic connections.

Lithography-based microfluidic platforms can also be designed to study two or more cell types, i.e. for co-culture of various cells, wherein medium conditions need to be standardized in order to study different cell types on the same platform. For instance, in 2017, Chen et al. developed a microfluidic device to study interaction between HUVECs and RPEs, under co-culture conditions. In this model, HUVECs and RPEs were separated by a porous membrane. It was observed that the cellular area and attachment to membranes were influenced by glucose and

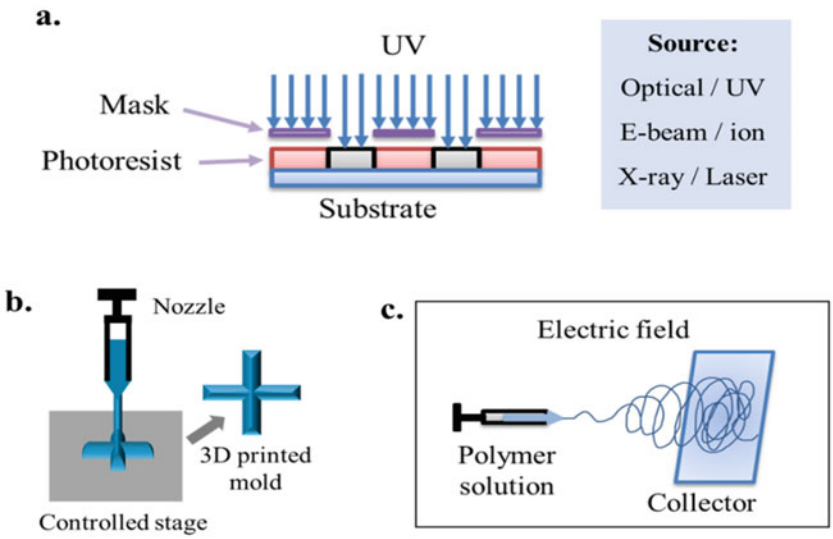

Fig. 17.5 Schematic representation of microfabrication methods: (**a**) lithography (**b**) 3D printing (**c**) electrospinning

oxygen levels in the culture. Recently, co-culture of monolayers of RPEs and HUVECs in micro-vessel form was established within a microfluidic device to study outer-BRB. Assays such as barrier permeability, fluorescence angiography, and optical coherence tomography for vascular structure of established co-culture systems were conducted [48]. Similarly, Yeste et al. demonstrated co-culture of human retinal endothelial cells (HREC), neuroblastoma cells, (SHSY5Y) and ARPE-19 cells, within a microfluidic platform [52]. In this model, highly interconnected micro-grooves promoted cell-cell interactions. Further, incorporation of electrodes within the chip enabled the measurement of transepithelial/transendothelial electrical resistance (TEER) and therefore exhibited potential of the on-chip model in evaluating cellular functions. Lithography has also been used to generate RO-on-chip models. For example, Achberger et al. fabricated microfluidic device to study interactions between hIPSCs-derived RPEs and RO. In this model, RPEs were cultured over a porous membrane for 24 h. RO encapsulated in hydrogel was then placed on the top of the RPE layer. This system helped in maintaining long-term culture for 21 days. Studies considering retinal-tissue are limited due to the inherent curved shape of the retina. In 2015, Dodson et al. obviated this limitation with the help of lithography-based microfluidics and developed a long-term culture platform for studying whole mice retina [42]. In the proposed RoC model, the retinal tissue was placed over a thin PDMS layer. When negative pressure was applied within the microfluidic channel below the thin layer, the retina tissue deformed and sealed the hole and became accessible for study. These holes acted as access points and could be used to study tissue or for reagent delivery. This model was anticipated

to help in investigations related to physiological and pathological conditions of the retinal tissue.

Recently, stereolithography-based 3D printers were used to fabricate a microfluidic platform to achieve long-term RO culture. In this method, polymer/material is sequentially deposited in the desired pattern, resulting in 3D structures (Fig. 17.5b). This method is used to fabricate accurate, complex, and durable microfluidic devices [53]. In this model, 6 × 5 array of chambers connected through microfluidic channels was fabricated using a 3D printer and used as a mold for soft lithography. This microfluidic platform was found to be suitable for long-term culture (~30 days) of stem cell-derived RO. Recently, attempts have been made to use electrospinning, in addition to lithography, in order to better mimic the in vivo conditions. Electrospinning is another technique which utilizes high voltage to draw fibres from the polymer solution or melt (Fig. 17.5c). Such fibrous matrices, varying from micron to nano scale, have been widely used to study the cell behaviour including alignment, migration, and differentiation [54]. For example, the electrospun nanofiber layer of PLA was embedded in place of a porous membrane. Both HUVECS and RPEs cells were found to be viable for 7 days [42]. Therefore, integration of various fabrication techniques can play a crucial role in RoCs that better mimic the retina and should be explored further.

Various other methods, such as, nanoimprinting, hot embossing, and micromachining injection molding, have also been widely employed for fabricating microfluidic devices for biomedical applications [55], but not yet explored for RoC applications. Reported studies show that microfluidic devices can play an important role in deciphering the role of various retina cells in both physiological and pathological conditions. However, major limitations in the development of RoC mimicking the retinal tissue lie in the recapitulation of complex in vivo coordination of cells for achieving cellular functions.

17.4.2 Characterization and Validation of RoCs

Since various cell lines can be used for in vitro retinal investigations, it is important to check cellular phenotypes cultivated in mono- and co-culture conditions. Validation assays are defined depending upon the known functions of the respective cells present in the in vivo retinal tissue. The biomarkers and validation assays studied in various RoCs have been summarized in Table 17.2.

17.5 Case Study of RoC

17.5.1 hIPSC-Derived RO Merging RoC Technology

In this study, Achberger et al. have discussed multi-layer tissue model generated using RoC technology. Pluripotent stem cells were used for differentiation into different retinal cell types on microfluidic platform. The resulting retinal tissue

Table 17.2 Biomarkers and validation assays in RoCs

Cells	Cellular functions	Biomarkers	Assays	Reference
Mouse RPCs	Cell migration	Pax6, Six3, OTX2, CRX	Boyden Chamber	Mishra et al. 2015 [16]
R28—RPCs	Synaptic connections	Vimentin, βIII-tubulin, PSD-95	Counting synaptic connections	Su et al. [47]
ARPE-19 and HUVEC	Cell-cell interaction	ZO-1	VEGF secretion	Chen et al. [48]
Fibroblast, HUVECs, ARPE-19	Mimic order of BRB	VE-cadherin IgG, ZO-1	Micro-vessel quality	Arık et al. 2021 [49]
ARPE-19, HREC, SHSY5Y	Cell interaction in barrier tissue	ZO-1	Permeability assay, TEER	Yeste et al. 2017 [52]
HiPSC-derived RO and RPEs	Interaction of RO with single cells	ZO-1, MITF, Pax-6, LAMP-2, AP2α	Phagocytosis assay, VEGF-A secretion, tunnel assay	Achberger et al. 2019 [39]

could mimic the in vivo anatomy and vascular system supplying nutrients and drug samples to the target cells. The hIPSC-derived RO were characterized for multi-layer-specific markers such as neural retinal marker (CHX10), rod (rhodopsin), cone (arrestin 3), s-cone (S-opsin), ganglion cells (BRN3B), amacrine cells (AP2α), bipolar cell (PKCα), müller glia cells (CRALBP), and outer limiting membrane (ZO-1). The polarization and functionality of RPE in RO were tested using PAX6 and MITF marker expression. Maturation and pigmentation of RPE were indicated using melanoma gp100 marker (also known as PMEL 17) providing conclusive evidence for functionality of RPE in its polarized state.

The developed RO on chip was tested for their applicability for drug development and toxicology studies using anti-malaria drug (chloroquine) and antibiotic (gentamicin). The study summarizes results from RO developed on chip: diversity of rod and cone, inner and outer segment formation, micro-physiology of RO on chip, specific marker expression and polarization of RPE in RoC, vasculature-mimicking channels, and understanding visual cycle retinal function through calcium ion flux imaging. Combinations of RO and RoC approaches used in this study successfully supported the development of various retinal cell types connected to the RPE layer. This 3D retinal model recapitulated the in vivo structural characteristics of the human retina and its behavioural response to light understanding demonstrated through functional RPE-photoreceptor model. This model was projected as a platform for testing diverse drugs meant for treating retinal diseases and for studying patient-specific treatment responses, during development of personalized therapies [39].

17.6 Advantages and Limitations of the RoC Technology

Despite significant progress in mimicking in vivo retinal features through implementation of sophisticated technologies, development of RoC is still faced with numerous challenges such as successful isolations of various human retinal cells, cellular viability in microfluidic devices, characterization of cells, and maintaining them as long-term culture. Some of the benefits and challenges presented by the RoC technology have been listed in Fig. 17.6. Adequate addressing of these challenges can accelerate the development of suitable RoC models that may have commercial implications in pre-clinical research pertaining to retinal therapeutics.

17.6.1 Advantages of RoC Technology

17.6.1.1 Accelerating Research

RoCs can bridge the gap between animal models and human clinical trials by enabling better understanding of the biological aspects of development, homeostasis, disease mechanism, signalling pathways, and providing a platform for screening of novel drugs. These models will accelerate research by helping in understanding retinal tissue biology using micro-engineered, compartmentalized fabrication methods that may facilitate interactions between retinal cell types [56].

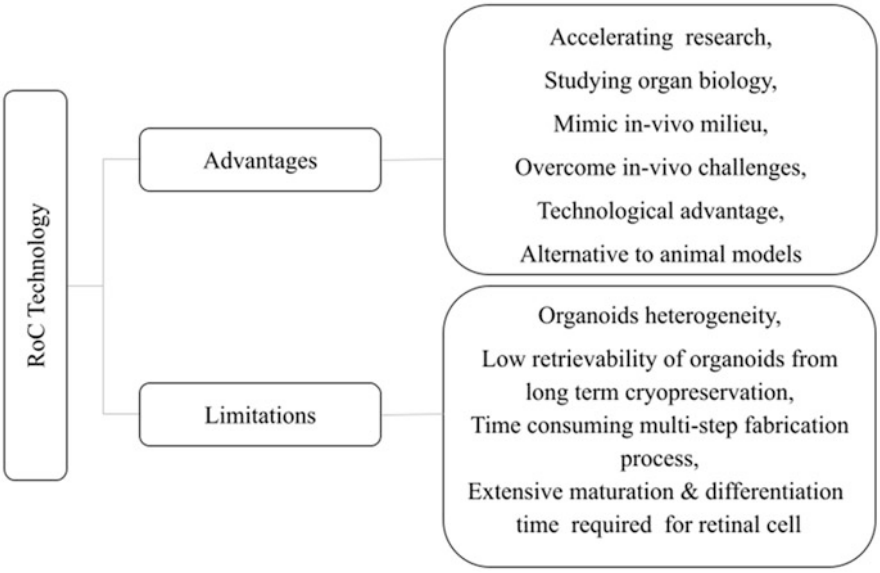

Fig. 17.6 Advantages and limitations of the in vitro RoC models

17.6.1.2 Overcoming In Vivo Challenges by Mimicking Its Niche in In Vitro Models

RoCs may be advanced into disease models with ability to replicate structural properties, the dynamic mechanical properties, and facilitate biochemical and microfluidic interactions between cells in the developed RO [57]. It can also support and provide insights into tissue differentiation, interactions between ECM proteins, spatio-temporal chemical gradients, and connective tissue microenvironment, so as to recapitulate the complex in vivo retinal anatomy [58].

17.6.1.3 Technological advantage

Advancements from 2D to 3D models and static to perfusion-based microfluidic devices and eventually to RoCs have been possible due to technological progress over time and deeper knowledge about the in vivo physiological and clinical aspects of tissues and diseases. Technologically advanced systems like the RoCs demand minimum usage of space and reduced volume of consumables and offer point-of-care devices, as well as mass production of organoids [59].

17.6.1.4 Alternative to Animal Models

Reduction in the need for animal trials and human clinical studies can enhance the cost-effectiveness of the RoC technology, particularly due to customized device fabrication under well-mimicked in vivo conditions [60]. Animal studies result in interspecies differences. Also, non-human primate models are easy to develop, but cannot mimic disease progression and are costly and met with ethical issues, which together reiterate the need for RoC technology [61].

17.6.2 Limitations of RO and RoC Technology

17.6.2.1 Difficult to Maintain Upon Long-Term Cryopreservation

The ROs are difficult to maintain upon retrieval after long-term cryopreservation. The retrieved ROs exhibit slow growth and reduced cellular viability with improper validation profiles when cultured again.

17.6.2.2 Absence or Patches of the RPE Layer

The mammalian retinal layer matures and polarizes into apical and basal sides, with microvilli on the apical side, and interacts and nurtures the photoreceptor cells. The apical RPE and photoreceptor junction is the site for signalling pathways and retinal disease development. These photoreceptor cells consist of rods and cones that participate in recycling of 11-trans retinal to 11-cis retinal pigment aiding visual cycle. The RoC organoids either completely lack the RPE layer or present it in patches, which is unsuitable for replicating similar retinal connections between the apical RPE and photoreceptors. This limits the suitability of these models for understanding the signalling pathways and retinal disease mechanisms [62].

17.6.2.3 Remodelling Vascularization

Recapitulating vascularization using this technology for important physiological interactions between mature photoreceptors and RPE cells is difficult to establish due to lack of a well-developed RPE layer or its presence as patches [63].

17.6.2.4 Heterogeneity of Organoids

The ROs exhibit heterogeneity with respect to their sizes, developmental stages, and proportion of various cell types, interfering with adequate characterization of drugs and establishing their screening profiles. The ROs generated using patient-derived samples consist of heterogeneous cell population, which interferes with final interpretations. This makes it difficult to associate the RO phenotype with a particular gene mutation or any other variation, thereby resulting in uncertainty during high-throughput drug screening [39].

17.6.2.5 Time-Consuming Multi-step Fabrication Process

Fabrication of RoC devices requires prolonged duration and manual operations to be performed. Device optimization may involve overcoming the leakage experienced with the laminar flow of the medium through the perfusion device. The perfusion flow of the medium also needs to be optimized to prevent air blockage or membrane blockage. The technology also presents technical errors such as back pressure development, AC/DC power system fluctuation, sterilization, seeding cells in 3D configurations, and operating the system under optimized mass transport and shear stress conditions [64, 65].

17.6.2.6 Extensive Maturation and Differentiation Time

Different retinal cell types require intensive and prolonged culturing so that the photoreceptor cells may become functional. Also, the light responsive RoC models require human iPSCs and face issues of limited scalability, response variability, tissue fidelity differences, etc. These challenges cannot be overcome with RoCs alone and requires their combination with RO, bioprinting, and miniaturized spinning bioreactor [66].

17.7 Impact of RoC Technology

17.7.1 Social Impact

The rapid progress in OoC technology over the past decades has enabled the creation of a wide spectrum of disease models, using microfluidic perfusion devices. The number of scientific papers describing the RoC technology has sharply increased, highlighting its growing importance and ability to remodel complex in vivo situations. With advancement in scientific research, RoC is transiting from fabrication of microfluidic devices for in vitro retinal layer development to differentiation of retinal cells into photoreceptor rods and cones, as well as tissue engineering. Understanding the societal needs and evolutionary trend of these models can help

in overcoming the future challenges in development of relevant in vitro human organotypic models [67]. The growth of these models has provided scientists with insights that were previously unachievable.

RoC technology has offered healthcare providers with an opportunity to conduct high-throughput screening of newer therapies and analyse their useful or adverse responses. Also, the potential of RoCs in development of personalized therapies cannot be ruled out if tissues are based on patient-specific cells as these RoCs will also represent the genetic make-up of patients [68].

17.7.2 Industry

RoC technology has demanded extensive collaborations between scientists having diverse complementary expertises, including in translational biology, cancer therapeutics, bio-banking, and drug discovery, as shown in Fig. 17.7. These platforms have created great opportunities for team work between researchers belonging to different fields like developmental biology, stem cell biology, material sciences, tissue, and bioengineering [69]. The microfluidic platforms have provided unique

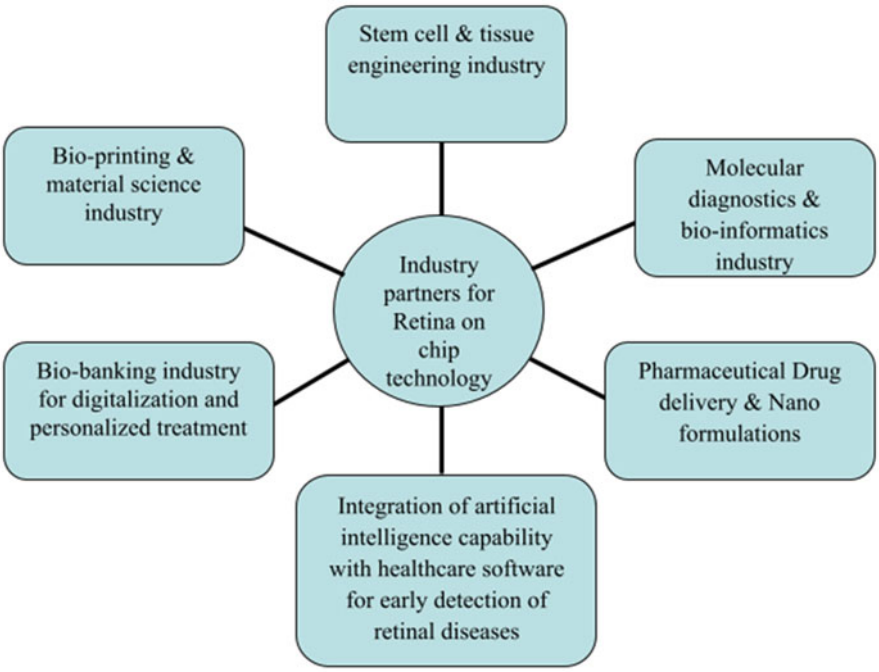

Fig. 17.7 Collaboration of industry partners involved with the RoC technology with scientists engaged in research related to stem cells and regenerative medicine, tissue engineering, molecular diagnostics, bioprinting, bio-banking, pharmaceuticals and nano-formulations, bioinformatics, and healthcare industries

tools for screening a wide range of novel drugs. The emerging capabilities of artificial intelligence and developments in the availability of healthcare software have enabled early detection and prevention of retinal diseases [70]. Machine learning and image recognition tools have opened up possibilities of conducting detailed and efficient investigations with organoids and the possibility of providing more relevant outputs via high-throughput drug screening [71].

Industrial partnering and government financial funding agencies have created resources for innovations and ideation in retinal disease models, using stem cells, iPSCs, tissue modelling and engineering, bioprinting for drug screening, and regenerative medicine [72]. Support includes kind consultation, access to optimization and validation platforms for drug screening, discounts on products and reagents, and other incentives. National Eye Institute (NEI) continues to build relationships between businesses and organizations interested in developing methodologies, media components, to enable increased scalability and reduction in variability experienced with ROs [73].

17.7.3 Economic Implications

The debilitating retinal degenerative diseases include AMD, glaucoma, diabetic retinopathy, and retinitis pigmentosa, which have been increasing annually with increasing age of global population [74, 75, Fig. 17.8]. This has raised concerns regarding maintenance of a healthy quality of life and the financial burden of patients for restoring their vision [74].

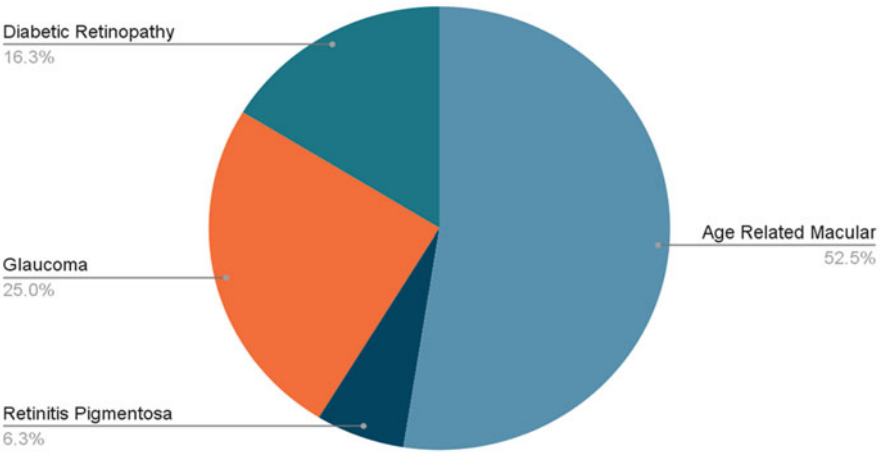

Fig. 17.8 Statistics of retinal degenerative diseases worldwide; 8.4 million people affected with age-related macular (AMD) disease, 4 million with glaucoma, 2.6 million with diabetic retinopathy, and over 1 million with retinitis pigmentosa, impacting the economy and quality of life

The global economic burden due to retinal degenerative diseases has increased from $2.3 trillion in 2010 to $2.8 trillion in 2020, primarily due to the high cost of treatments and other medical expenses [74].

Novel drugs for retinal diseases require extensive approvals for clinical trials at various stages of its manufacturing and testing with less chances of successful release of the drug into the market for disease treatment. Major drugs fail in late stages of clinical trial due to lower reproducibility and reduced efficacy in humans, as against animal investigations. This has further affected the possibility of affordable drugs for retinal diseases. The development of RoCs will seal the gap between animal and clinical investigations, leading to more drug approvals by regulatory agencies and reduce the losses incurred by the healthcare industry. The 3D mini-organoid based on scaffolds can be a game changer in RoCs, alluring the healthcare and pharma market for investing further in novel drug development and development of personalized medicines [76].

17.8 Future Scope of RoC Technology

Till date, complete eye transplant is awaiting success and is met with challenges due to complex physiology and anatomy of the eye and its neuronal brain connectivity. Vision restoration with prosthetic devices such as the Argus II system enables patients to perceive their surroundings, see objects as black and white and read large printed fonts in some cases. Development of in vitro RoC technology ensures a future for developing genetically matched donor organs for specific recipients, overcoming the need for donor availability search [77]. Gene therapies using adeno-associated viruses (AAV) are among the most promising strategies to treat or even cure hereditary and acquired retinal diseases. However, the development of new efficient AAV vectors is slow and costly, largely because of the lack of suitable non-clinical models. By precisely recreating structure and function of human tissues, hIPSCs-derived RO could become an essential part of the test cascade addressing translational aspects [77]. OoC technology provides the capability to recapitulate micro-physiological tissue environments as well as a precise control over structural and temporal parameters. The recent RoCs that merge organoids and OoC technologies have enabled accurate analysis of efficacy, kinetics, and cell tropism of seven first- and second-generation AAV vectors. The data demonstrate the potential of iPSC-based OoC models as the next generation of screening platforms for future gene therapeutic studies [39].

17.9 Conclusion

This chapter summarizes the developments that have been made in microfluidic perfusion-based RoC devices for use as in vitro disease organoid models and for development of novel drugs. These may be based either on iPSCs or patient-derived cells. Also, integration of organoids with OoCs is the future of RoCs, which in

conjunction with bioprinting may facilitate development of interventions for restoring vision. The RoC technology will form an integral part of research to develop ROs as disease models. Retinal models developed with patient-specific cells may lead to screening of chemotherapy drugs at clinical trial stage.

References

1. Cora V, Haderspeck J, Antkowiak L, Mattheus U, Neckel PH, Mack AF, Bolz S, Ueffing M, Pashkovskaia N, Achberger K, Liebau S (2019) A cleared view on retinal organoids. Cells 8(5): 391. https://doi.org/10.3390/cells8050391
2. Eiraku M, Takata N, Ishibashi H, Kawada M, Sakakura E, Okuda S, Sekiguchi K, Adachi T, Sasai Y (2011) Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature 472(7341):51–56. https://doi.org/10.1038/nature09941
3. Zhong X, Gutierrez C, Xue T, Hampton C, Vergara MN, Cao L-H, Peters A, Park TS, Zambidis ET, Meyer JS et al (2014) Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. Nat Commun 5:4047. [CrossRef]-retina
4. Shafaie S, Hutter V, Cook MT, Brown MB, Chau DYS (2016) In vitro cell models for ophthalmic drug development applications. Biores Open Access 5(1):94–108. https://doi.org/10.1089/biores.2016.0008. Published online 2016 Apr 1
5. Ubels JL, Clousing DP (2005) In vitro alternatives to the use of animals in ocular toxicology testing. Ocul Surf 3(3):126–142. https://doi.org/10.1016/s1542-0124(12)70195-7
6. Toropainen E (2007) Corneal Epithelial Cell Culture Model for Pharmaceutical Studies. University of Kuopio distributor: Kuopio University Library
7. Nakano T, Ando S, Takata N, Kawada M, Muguruma K, Sekiguchi K, et al (2012) Self-formation of optic cups and storable stratified neural retina from human ESCs. Cell Stem Cell [Internet]. [cited 2021 Aug 31];10(6):771–85. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1934590912002421
8. Soo JY, Jansen J, Masereeuw R, Little MH (2018) Advances in predictive in vitro models of drug-induced nephrotoxicity. Nat Rev Nephrol 14(6):378–393. https://doi.org/10.1038/s41581-018-0003-9. PMID: 29626199; PMCID: PMC6013592
9. Achberger K, Haderspeck JC, Kleger A, Liebau S (2019) Stem cell-based retina models. Adv Drug Deliv Rev 140:33–50
10. Wikswo JP (2014) The relevance and potential roles of microphysiological systems in biology and medicine. Exp Biol Med 239(9):1061–1072
11. Peiffer RL Jr, Pohm-Thorsen L, Corcoran K (1994) Models in ophthalmology and vision research. In: The biology of the laboratory rabbit, pp 409–433. https://doi.org/10.1016/B978-0-12-469235-0.50025-7. Epub 2013 Oct 21
12. Volland S, Esteve-Rudd J, Hoo J, Yee C, Williams DS (2015) A comparison of some organizational characteristics of the mouse central retina and the human macula. PLoS One 10(4):e0125631. https://doi.org/10.1371/journal.pone.0125631
13. Zhang B, Korolj A, Lai BF, Radisic M (2018) Advances in organ-on-a-chip engineering. Nat Rev Mater 3(8):257–278
14. Puleo CM, Ambrose WM, Takezawa T, Elisseeff J, Wang TH (2009) Integration and application of vitrified collagen in multilayered microfluidic devices for corneal microtissue culture. Lab Chip 9(22):3221–3227
15. Chen LJ, Ito S, Kai H, Nagamine K, Nagai N, Nishizawa M, Abe T, Kaji H (2017) Microfluidic co-cultures of retinal pigment epithelial cells and vascular endothelial cells to investigate choroidal angiogenesis. Sci Rep 7(1):1–9
16. Mishra S, Thakur A, Redenti S, Vazquez M (2015) A model microfluidics-based system for the human and mouse retina. Biomed Microdevices 17(6):107

17. Mathur A, Loskill P, Shao K, Huebsch N, Hong S, Marcus SG, Marks N, Mandegar M, Conklin BR, Lee LP, Healy KE (2015) Human iPSC-based cardiac microphysiological system for drug screening applications. Sci Rep 5(1):1–7
18. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE (2010) Reconstituting organ-level lung functions on a chip. Science 328:1662–1668
19. Wilmer MJ, Ng CP, Lanz HL, Vulto P, Suter-Dick L, Masereeuw R (2016) Kidney-on-a-chip technology for drug-induced nephrotoxicity screening. Trends Biotechnol 34(2):156–170
20. Nakao Y, Kimura H, Sakai Y, Fujii T (2011) Bile canaliculi formation by aligning rat primary hepatocytes in a microfluidic device. Biomicrofluidics 5(2):022212
21. Clevers H (2016) Modeling development and disease with organoids. Cell 165(7):1586–1597. https://doi.org/10.1016/j.cell.2016.05.082
22. Clevers HC (2019) Organoids: avatars for personalized medicine. Keio J Med 68(4):95. https://doi.org/10.2302/kjm.68-006-ABST
23. De Crignis E, Hossain T, Romal S, Carofiglio F, Moulos P, Khalid MM et al (2021) Application of human liver organoids as a patient-derived primary model for HBV infection and related hepatocellular carcinoma. eLife [Internet] 10:e60747. [cited 2021 Aug 30]
24. Willoughby CE, Ponzin D, Ferrari S, Lobo A, Landau K, Omidi Y (2010) Anatomy and physiology of the human eye: effects of mucopolysaccharidoses disease on structure and function - a review: Anatomy and physiology of the eye. Clin Exp Ophthalmol 38:2–11
25. Gill JS, Georgiou M, Kalitzeos A, Moore AT, Michaelides M (2019) Progressive cone and cone-rod dystrophies: clinical features, molecular genetics and prospects for therapy. Br J Ophthalmol 103(5):711–720
26. Kalargyrou AA, Pearson RA (2020) Photoreceptor transplantation: re-evaluating the mechanisms that underlie rescue. In: Fritzsch B (ed) The senses: a comprehensive reference, 2nd edn. Elsevier, Oxford, pp 614–629. [Internet] [cited 2021 Oct 4]
27. Susan De Remer. Layers of the Retina. Discovery Eye Foundation [Internet]. [cited 2021 Oct 5]. Available from: https://discoveryeye.org/layers-of-the-retina
28. Ajioka I, Martins RAP, Bayazitov IT, Donovan S, Johnson DA, Frase S et al (2007) Differentiated horizontal interneurons clonally expand to form metastatic retinoblastoma in mice. Cell 131:378–390. https://doi.org/10.1016/j.cell.2007.09.036
29. Ueno S, Nishiguchi KM, Tanioka H, Enomoto A, Yamanouchi T, Kondo M et al (2013) Degeneration of retinal ON bipolar cells induced by serum including autoantibody against TRPM1 in mouse model of paraneoplastic retinopathy. PLoS One 8(11):e81507
30. Nagashima K, Kikuchi F, Suzuki Y, Abe T (1981) Retinal amacrine cell involvement in Tay-Sachs disease. Acta Neuropathol 53(4):333–336
31. External limiting membrane - Membranum limitans externum [Internet]. IMAIOS. Available from: https://www.imaios.com/en/e-Anatomy/Anatomical-Parts/External-limiting-membrane
32. Macular Hole | National Eye Institute [Internet]. [cited 2021 Oct 18]. Available from: https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/macular-hole
33. Retinal Tears - Patients - The American Society of Retina Specialists [Internet]. [cited 2021 Oct 5]. Available from: https://www.asrs.org/patients/retinal-diseases/26/retinal-tears
34. Retinal detachment - Diagnosis and treatment - Mayo Clinic [Internet]. [cited 2021 Oct 18]. Available from: https://www.mayoclinic.org/diseases-conditions/retinal-detachment/diagnosis-treatment/drc-20351348
35. Blair K, Czyz CN (2021) Retinal Detachment [Internet]. StatPearls [Internet]. StatPearls Publishing. [cited 2021 Oct 4]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK551502/
36. Vujosevic S, Midena E (2013) Retinal layers changes in human preclinical and early clinical diabetic retinopathy support early retinal neuronal and Müller cells alterations. J Diabet Res 2013:e905058

37. Diagnosis and treatment - Mayo Clinic [Internet]. [cited 2021 Oct 18]. Available from: https://www.mayoclinic.org/diseases-conditions/diabetic-retinopathy/diagnosis-treatment/drc-20371617?p=1
38. Macular Hole: Symptoms, Treatments [Internet]. Cleveland Clinic. [cited 2021 Oct 4]. Available from: https://my.clevelandclinic.org/health/diseases/14208-macular-hole
39. Achberger K, Probst C, Haderspeck J, Bolz S, Rogal J, Chuchuy J et al (2019) Human Retina-on-a-Chip: merging organoid and organ-on-a-chip technology to generate complex multi-layer tissue models in a human retina-on-a-chip platform. elife 8:e46188
40. Fligor CM, Langer KB, Sridhar A, Ren Y, Shields PK, Edler MC et al (2018) Three-dimensional retinal organoids facilitate the investigation of retinal ganglion cell development, organization and neurite outgrowth from human pluripotent stem cells. Sci Rep 8(1):14520
41. Koens R, Tabata Y, Serrano JC, Aratake S, Yoshino D, Kamm RD et al (2020) Microfluidic platform for three-dimensional cell culture under spatiotemporal heterogeneity of oxygen tension. APL Bioeng 4(1):016106
42. Dodson KH, Echevarria FD, Li D, Sappington RM, Edd JF (2015) Retina-on-a-chip: a microfluidic platform for point access signaling studies. Biomed Microdevices 17(6):114
43. Retina-on-a-chip provides powerful tool for studying eye disease - Science Daily [Internet]. [cited 2021 Oct 5]. Available from: https://www.sciencedaily.com/releases/2019/08/190827095104.htm
44. Chung M, Lee S, Lee BJ, Son K, Jeon NL, Kim JH (2018) Wet-AMD on a chip: modeling outer blood-retinal barrier in vitro. Adv Healthc Mater 7(2):1700028
45. Peter Loskill Lab | Retina on Chip [Internet]. [cited 2021 Oct 5]. Available from: http://loskill-lab.com/retinachip.html
46. Pattanayak P, Singh SK, Gulati M, Vishwas S, Kapoor B, Chellappan DK et al (2021) Microfluidic chips: recent advances, critical strategies in design, applications and future perspectives. Microfluid Nanofluid 25(12):99
47. Su P-J, Liu Z, Zhang K, Han X, Saito Y, Xia X et al (2015) Retinal synaptic regeneration via microfluidic guiding channels. Sci Rep 5(1):13591
48. Chen L-J, Ito S, Kai H, Nagamine K, Nagai N, Nishizawa M et al (2017) Microfluidic co-cultures of retinal pigment epithelial cells and vascular endothelial cells to investigate choroidal angiogenesis. Sci Rep 7(1):3538
49. Arık YB, Buijsman W, Loessberg-Zahl J, Cuartas-Vélez C, Veenstra C, Logtenberg S et al (2021) Microfluidic organ-on-a-chip model of the outer blood–retinal barrier with clinically relevant read-outs for tissue permeability and vascular structure. Lab Chip 21(2):272–283
50. Chung BG, Choo J (2010) Microfluidic gradient platforms for controlling cellular behavior. Electrophoresis 31(18):3014–3027
51. Fiorini GS, Chiu DT (2005) Disposable microfluidic devices: fabrication, function, and application. BioTechniques 38(3):429–446
52. Yeste J, García-Ramírez M, Illa X, Guimerà A, Hernández C, Simó R et al (2017) A compartmentalized microfluidic chip with crisscross microgrooves and electrophysiological electrodes for modeling the blood–retinal barrier. Lab Chip 18(1):95–105
53. Soft lithography for micro- and nanoscale patterning | Nature Protocols [Internet]. [cited 2021 Oct 28]. Available from:https://www.nature.com/articles/nprot.2009.234?WT.feed_name=subjects_soft-lithography
54. Prabhakar P, Sen RK, Dwivedi N, Khan R, Solanki PR, Srivastava AK et al (2021) 3D-printed microfluidics and potential biomedical applications. Front Nanotechnol 3:6
55. Agarwal S, Wendorff JH, Greiner A (2008) Use of electrospinning technique for biomedical applications. Polymer 49(26):5603–5621
56. Xue Y, Seiler MJ, Tang WC, Wang JY, Delgado J, McLelland BT et al (2021) Retinal organoids on-a-chip: a micro-millifluidic bioreactor for long-term organoid maintenance. Lab Chip 21(17):3361–3377

57. Chuchuy J, Rogal J, Ngo T, Stadelmann K, Antkowiak L, Achberger K et al (2021) Integration of electrospun membranes into low-absorption thermoplastic organ-on-chip. ACS Biomater Sci Eng 7(7):3006–3017
58. Moraes C, Mehta G, Lesher-Perez SC, Takayama S (2012) Organs-on-a-Chip: a focus on compartmentalized microdevices. Ann Biomed Eng 40:1211–1227
59. Van der Meer AD, Van den Berg A (2012) Organs-on-chips: breaking the in vitro impasse. Integr Biol (Camb) 4:461–576. https://doi.org/10.1039/c2ib00176d
60. Huh D, Hamilton GA, Ingber DE (2011) From 3D cell culture to organs-on-chips. Trends Cell Biol 21:745–754. https://doi.org/10.1016/j.tcb.2011.09.005
61. Polini A, Prodanov L, Bhise NS, Manoharan V, Dokmeci MR, Khademhosseini A (2014) Organs-on-a-chip: a new tool for drug discovery. Expert Opin Drug Discov 9:335–352. https://doi.org/10.1517/17460441.2014.886562
62. Singh RK, Nasonkin IO (2020) Limitations and promise of retinal tissue from human pluripotent stem cells for developing therapies of blindness. Front Cell Neurosci 14:179
63. Capowski EE, Samimi K, Mayerl SJ, Phillips MJ, Pinilla I, Howden SE, Saha J, Jansen AD, Edwards KL, Jager LD et al (2019) Reproducibility and staging of 3D human retinal organoids across multiple pluripotent stem cell lines. Development 146:dev171686
64. Zhang Z, Xu Z, Yuan F, Jin K, Xiang M (2021) Retinal organoid technology: where are we now? Int J Mol Sci 22:10244. https://doi.org/10.3390/ijms221910244
65. Kim L, Toh YC, Voldman J, Yu H (2007) A practical guide to microfluidic perfusion culture of adherent mammalian cells. Lab Chip 7(6):681–694
66. Wright CB, Becker SM, Low LA, Tagle DA, Paul A (2020) Sieving. improved ocular tissue models and eye-on-a-chip technologies will facilitate ophthalmic drug development. J Ocul Pharmacol Ther 36(1):25–29
67. Wang Z, He X, Qiao H, Chen P (2019) Global trends of organoid and organ-on-a-chip in the past decade: a bibliometric & comparative study. Tissue Eng. https://doi.org/10.1089/ten.TEA.2019.0251
68. Cai S, He L, Zheng F, Kong F, Dao M, Karniadakis GE, Suresh S (2021) Artificial intelligence velocimetry and microaneurysm-on-a-chip for three-dimensional analysis of blood flow in physiology and disease. Proc Natl Acad Sci U S A 118(13):e2100697118. https://doi.org/10.1073/pnas.2100697118
69. Hailstone M, Waithe D, Samuels TJ, Yang L, Costello I, Arava Y, Robertson E, Parton RM, Davis I (2020) CytoCensus, mapping cell identity and division in tissues and organs using machine learning. elife 9:e51085
70. Mazerik JN, Becker S, Sieving PA (2018) 3-D retina organoids: building platforms for therapies of the future. Cell Med 10:1–6
71. Wright CB, Becker SM, Low LA, Tagle DA, Sieving PA (2020) Improved ocular tissue models and eye-on-a-chip technologies will facilitate ophthalmic drug development. J Ocul Pharmacol Ther 36(1):25–29. https://doi.org/10.1089/jop.2018.0139
72. Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, Das A, Jonas JB, Keeffe J, Kempen JH, Leasher J, Limburg H, Naidoo K, Pesudovs K, Silvester A, Stevens GA, Tahhan N, Wong TY, Taylor HR (2017) Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. Lancet Glob Health 5(12):e1221–e1234
73. Wong CH, Siah KW, Lo AW (2019) Estimation of clinical trial success rates and related parameters. Biostatistics 20(2):273–286
74. Aasen DM, Vergara MN (2020) New drug discovery paradigms for retinal diseases: a focus on retinal organoids. J Ocul Pharmacol Ther 36(1):18–24. https://doi.org/10.1089/jop.2018.0140
75. Gordois A, Cutler H, Pezzullo L et al (2012) An estimation of the worldwide economic and health burden of visual impairment. Glob Public Health 7(5):465–481. https://doi.org/10.1080/17441692.2011.634815
76. Bleijs M, van de Wetering M, Clevers H, Drost J (2019) Xenograft and organoid model systems in cancer research. EMBO J 38(15):e101654. https://doi.org/10.15252/embj.2019101654

77. Achberger K, Cipriano M, Düchs M, Schön C, Michelfelder S, Stierstorfer B, Lamla T, Kauschke SG, Chuchuy J, Roosz J, Mesch L, Cora V, Pars S, Pashkovskaia N, Corti S, Kleger A, Kreuz S, Maier U, Liebau S, Loskill P (2021) Human stem cell-based retina-on-chip as new translational model for validation of AAV retinal gene therapy vectors. Stem Cell Rep. https://doi.org/10.1101/2021.03.02.433550

Heart-on-a-Chip

18

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Abstract

A heart-on-a-chip is a microfluidic device that mimics the structural and functional characteristics of the heart by culturing cardiomyocytes on a microfluidic platform. The device can be used for several applications like studying the physiology of cells, analyzing the effect of various drugs and chemicals on

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heart cells, or evaluating the cell response to different stimulations. This chapter begins with a brief discussion on the need for heart-on-a-chip devices followed by an explanation on the physiological environment and functions of the heart cells. The cell biological requirements along with the design, simulation, and microfabrication of heart cells on chip are then discussed. The different optical and electrical methods for studying the cultured cardiomyocytes are also briefed. The chapter concludes by addressing some of the current challenges and future scope for heart-on-a-chip devices. These devices have the potential to replace the conventional animal model studies and provide better insights to disease modelling and cardiotoxicity studies by closely mimicking in vivo conditions on a microfluidic platform.

Keywords

Heart-on-a-chip · Cardiac disease modelling · 3D cardiomyocyte culture · Action potential · Contractility

18.1 Introduction

A heart-on-a-chip is a microfluidic device used to mimic the physiological conditions and functioning of cardiomyocytes by culturing cardiac cells in it. Advancements in cellular biology and micro-electro-mechanical systems led to interesting, cutting-edge research, which evolved heart-on-a-chip devices (Fig. 18.1). The chip typically houses multiple microchannels and reservoirs used to culture heart cells and deliver various nutrients necessary for the growth of the heart cells inside the microfluidic chip. The precise delivery of reagents for the controlled growth of heart cells is achieved using microfluidic components like micromixers, pressure sensors, flow sensors, microvalves, and actuators [Automated and programmable electromagnetically actuated valves for microfluidic applications] [Enhancement in mixing efficiency by ridges in straight and meander microchannels]. It is monitored in real-time using biosensors and imaging devices. The development of a heart-on-a-chip platform is highly interdisciplinary. It integrates cell biology, nanotechnology, microfabrication, and microelectronics to monitor the mechanical and electrical signals of the engineered cardiac platform. Microfluidic models for heart-on-a-chip can facilitate high-throughput analysis and precisely evaluate cardiac function, providing ample opportunities to assess physiological, pathological, and pharmacokinetics of the cardiac cells.

18.2 Need for Heart-on-a-Chip Models

Cardiovascular diseases are one of the leading causes of death and disability in the world. Thus, there is a need for developing novel drugs for the treatment and prevention of such diseases. Unfortunately, most drug discovery methods fail in

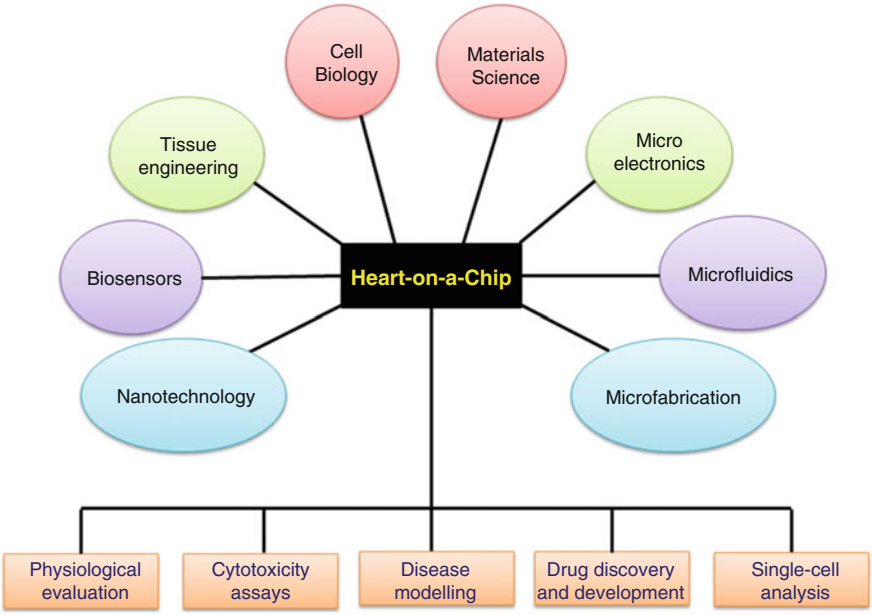

Fig. 18.1 Schematic diagram showing the interdisciplinary nature of the heart-on-a-chip devices and their potential applications

the evaluation period due to some cardiotoxic effects the formulated drug can have on the heart. The majority of the challenges faced by existing drug discovery methods are the poor prediction of the drug performance in humans, even if the drug is promising in animal studies. Also, certain drugs might respond differently to different organisms and might be limited to certain people, for instance, people of a particular age group. Therefore, an efficient model must mimic the human dynamics and environment as close as possible affordably and quickly. The existing methods are primarily lengthy and expensive. Hence, there is a growing need for methods that can aid the drug discovery process by performing a practical risk analysis of the formulated drug in a cost-effective but efficient and effective manner. A report that analysed the drug discovery pipeline of AstraZeneca from 2005 to 2010 showed that around 82% of the drugs discovered had to be closed down at preclinical trials due to safety concerns, and among them, 17% accounted for heart failures [1]. In these situations, organ-on-a-chip models can be promising as they can provide close resemblances to the human environment and can be used for better evaluation of drugs. They could revolutionize the identification of drugs, performance analysis, safety, and efficacy evaluation and cater to personalized needs.

Moreover, these devices can study the cell responses when exposed to different mechanical strains, shear stresses, and electrical stimulation, providing significant information for cellular studies. They can also be used to recapitulate in vivo functions like cell-cell interactions and cell-matrix interactions. Additionally, normal

and pathological conditions like normoxia and hypoxia can be modelled to study cellular behaviour.

18.3 Physiological Environment and Functions of the Heart

The heart is the centre of the human circulatory system, with four distinct chambers: two atria and two ventricles. Deoxygenated blood from different body parts is collected in the right atrium, passes to the right ventricles, and pumps to the lungs for oxygenation. Oxygenated blood from the lung reaches the left atrium and ventricle and pumps across the body through the arterial system. Interatrial and interventricular septa separate the right and the left chamber to prevent mixing oxygenated and deoxygenated blood.

The heart has an outer membranous covering termed pericardium. The heart muscle has the outer visceral pericardium/epicardium, middle layer myocardium, and inner layer endocardium. Epicardium is the thin covering of the heart made up of epithelial cells. Epicardial cells are activated in response to injury to the heart and differentiate into different types of cells. In addition, epicardial cells are responsible for paracrine signalling to the myocardium in response to cardiac injury. Highly organized parallel sheets of cardiomyocytes along with intercalated cardiac fibroblasts are present in the myocardium. Myocytes are loaded with plenty of mitochondria and are metabolically very active to provide energy to sustain cardiac muscle contraction. The heart is abundantly perfused with microcapillaries lined by endothelial cells. Cardiac fibroblasts and the extracellular matrix, composed of Type-I and Type-III collagen, provide a fibrous skeleton with a viscoelastic, stress-tolerant environment enabling smooth contraction and relaxation of muscle cells. The endocardium is the innermost layer of the heart lining the chambers, and the cells are very similar to endothelial cells.

Cardiomyocytes account for about 25–35% of all heart cells and have contractile functionality. In addition, cardiomyocytes function in collaboration with non-myocytes. Non-myocytes include: (1) Cardiac fibroblasts for remodelling of the interstitial space; (2) Endothelial cells and smooth muscle cells in the capillaries; (3) Immune cells; and (4) Neuronal cells for autonomic regulation. Cardiac fibroblasts are the significant non-myocyte present in the heart responsible for extracellular remodelling. The heart has a small population of B and T cells in addition to tissue-resident macrophages. The heart has also a substantial number of endothelial cells lining the capillaries and has a vital role in cellular perfusion (Fig. 18.2).

Several cardiac cellular models are available for disease modelling and drug screening, each with advantages and disadvantages. Most of these traditional models use classical 2D cultures. In 2D cultures, cardiomyocytes exhibit low electrical action potentials, are mononucleated, with disorganized sarcomere arrangement, having fetal-like metabolism, characteristic of an immature phenotype, and thus do not reflect the contractile properties and physiological phenotypes of adult cardiomyocytes. Initially, because of the availability, most of the heart-on-a-chip

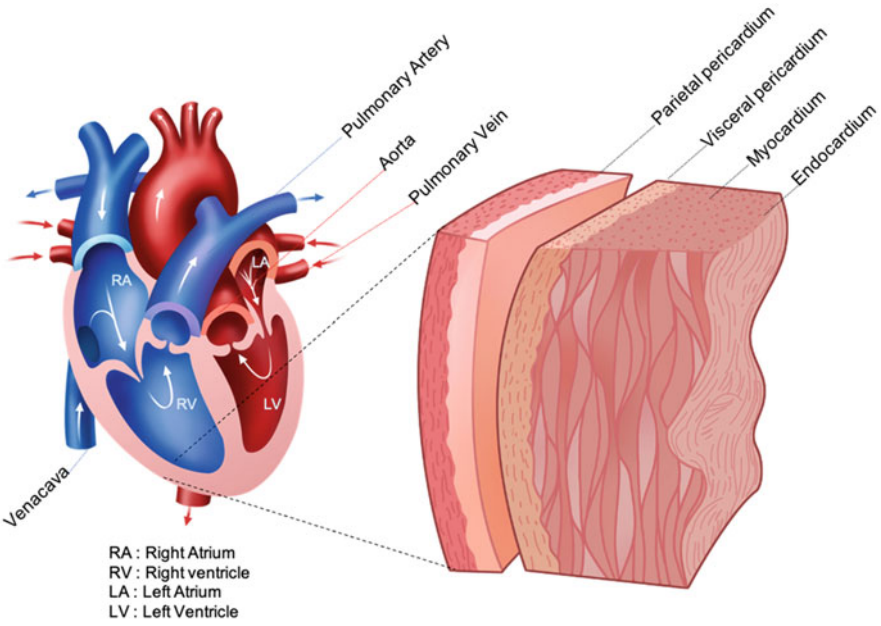

Fig. 18.2 Human Heart: Anatomy, circulation, and different layers

systems utilize neonatal rat cardiomyocytes that form muscle bundles and perform functions, but are of non-human origin [2]. Because of the species difference, the functional factors were different from human tissue.

Primary cardiomyocytes from human tissue are most preferred in terms of functionality. However, cells obtained were mainly from patients with heart failure, and thus donor-to-donor variability occurs, which hampers the utility of the model. In addition, for disease modelling and drug screening/sensitivity, healthy mature human cells are preferred over unhealthy cells or cells from other species. Furthermore, primary cardiomyocytes do not proliferate well in vitro and thus cannot be cultured indefinitely. Hence, more robust cell sources that ensure stable and functionally mature phenotype and having constant availability are required for heart-on-a-chip applications.

With the advent of induced pluripotent stem cell technology, different somatic cells can be derived from human-induced pluripotent stem cells (hiPSCs) efficiently and can be used to model cardiac tissue. The advantage of hiPSC is that gene-editing technology such as CRISPR-Cas technology can be combined with iPSC technology to produce specific disease mutations or restore mutations, thus accurately modelling cardiac diseases [3]. hiPSCs can be derived from readily available patient cells such as the blood or the skin, and many refined protocols are now available to differentiate different cardiac lineages. The major limitation of iPSC-derived cardiomyocytes is their immature nature. Different modifications are underway to ensure the maturity

of cardiomyocytes derived from iPSCs (discussed below). These cells are promising to establish a physiologically relevant in vitro 3D heart on a chip model system.

18.4 Cell Biological Requirements of a Heart-on-a-Chip

Heart cells in a microfluidic device should closely mimic those in their adult microenvironment as physiological tissue for disease modelling and drug development.

18.4.1 Requirement of Anisotropy and Mechanical Stimulus

Adult cardiomyocytes are terminally differentiated muscular cells connected by gap junctions. The contractile structure in a cardiac muscle (CM) is the myofibril, and the contractile function is possible because of the organized parallel arrangement of myofibril bundles. This organized arrangement helps transmit electrical impulses between cells and primarily along the long axis, creating synchronized contraction and relaxation. An increase and decrease in intracellular Ca^{2+} ions characterize the contraction and relaxation of cardiomyocytes. The heart muscle during development and in the adult is exposed to continuous mechanical load. Therefore, introducing mechanical overload by applying cyclic stretch in the stretchable chip helped accurately model stress-induced changes in cardiac tissue [4]. In addition, providing physical constraints, bio wires in 3D heart tissue, also results in increased myofibril organization and enhanced conduction velocity in 3D heart tissue [5]. Furthermore, studies show that cyclic stretch and electrical stimulation enhance the contraction and maturity of cardiomyocytes [6, 7]. Also, mechanical loading by cyclic stretch in human iPSC cardiac tissue resulted in improved twitch force, elastic modulus, sarcomere length, and molecular signature characteristic of enhanced maturity [8]. Thus, directional alignment of cardiac muscle can be attained in the heart-on-a-chip by having mechanical overload or physical constraints such as wires/posts or having cells grown in stretchable substrates.

18.4.2 Requirement of Electrical Stimulation

An intrinsic electrophysiological system regulates the pumping action of heart, starting from the sinoatrial node (SA node). The SA node sends electrical impulses to the atrioventricular node (AV node), then through the Purkinje fibres, impulses reach the apex of the heart. Contraction begins from the apex of the heart. SA node is the natural pacemaker of the heart, and it generates action potentials 100 times per minute in the absence of parasympathetic or sympathetic stimulation and other hormonal controls. Electrical stimulation leads to the depolarization of cardiac muscles, which results in synchronized contractions and relaxations.

Electrical stimulation of the anisotropic cardiac muscle bundle is an essential requirement in the heart on a chip. Several different techniques have been reported to enhance the alignment of the cultured heart cells. Integrated electrodes in a chip model can provide electrical stimulation, and studies showed that electric stimulation enhances the maturity of cardiomyocytes in 3D systems [9, 10].These electrical signals can be applied continuously or as pulses that mimic the native myocardium in different waveforms like square or sinusoidal using a potentiostat. One of the common methods used presently is to use a pair of carbon or platinum electrodes and apply an electric field between them, where the cells would be present. To improve the precision, microelectrode arrays have been developed, which allows for resolution up to a single cell. 3D electrodes like interdigitated electrodes have also been developed to provide electrical stimulation to models having thick tissues. Furthermore, the electrical properties of the scaffolds can be modified using nanomaterials like carbon nanotubes and gold nanowires to improve the electrical conduction in the system. The contractile measurements of the cardiomyocytes can be acquired using a biosensing board like OpenBCICyton Biosensing Board.

Optical methods have also been developed to stimulate the cardiomyocytes. Parameswaran et al. had demonstrated a moving low irradiance laser input for stimulating the cultured rat cardiomyocytes [11]. A pre-stimulus frequency of the cells is identified, and the cells are stimulated using programmed cycles of laser pulses. A feedback mechanism for monitoring the post-stimulus frequency is also present to achieve the target frequency for beating cells. Orlova et al. had presented electrospun nanofibers meshes of polymethylglutarimide (PMGI) as scaffolds for engineered cardiac cells [12]. This method could preserve the 3D architecture as well as the structural anisotropy of the cultured cardiomyocytes. Additionally, surface coatings of laminin and fibronectin have been employed to provide an environment similar to that in vivo. For the proper electrical coupling, orchestrated functioning of heterogeneous cell populations such as fibroblast and endothelial cells in the heart is also required [13, 14]. So, including the essential non-myocyte cell in the heart on a chip arrangement provides proper electrical propagation, enabling regular contraction and relaxation of the heart muscle.

18.4.3 Requirement of Non-myocytic Cells and ECM Interactions

As mentioned earlier, non-myocytic cells enhance the electrical coupling of cardiomyocytes through gap junctions and are essential for the proper conduction of electric signals enabling synchronous contraction and relaxation of cardiac muscle fibres [15, 16]. Furthermore, non-myocytes such as mesenchymal stem cells or cardiac fibroblasts scaffolds are known to enhance the regenerative potential of cardiac tissue [17]. Thus, they were used in different cardiac tissue engineering models to help tissue formation and remodelling [18]. Studies clearly show that the presence of cardiac fibroblasts within the microenvironment can significantly enhance the structural properties and tissue function [19–25].

The percentage of cardiac fibroblasts and total cell density also determine the optimal contractile function of the tissue [26]. In addition, cardiac fibroblasts act as the fibrous skeleton and produce extracellular matrix promoting tissue structuring and remodelling [27]. The extracellular matrix (ECM) environment plays a significant role in cell behaviour, functionalities, and viability of cardiac cells [28]. Heart-on-a-Chip models use extracellular matrix proteins such as collagen, matrigel, fibronectin, and fibrin as geometric cell-adhesion coatings [5, 29, 30].

18.4.4 Requirement of High Oxygen Level and Removal of Waste Products from Cells

Heart purely depends on aerobic metabolism where mitochondria have to maintain a high oxygen level for oxidative phosphorylation, and thus the oxygen demand in each cell is increased. This increased oxygen demand is met by capillary perfusion to avoid hypoxia and to maintain myocardial tissue integrity. In addition, the cardiac muscle beats continuously to pump blood. Thus, the energy demand in myocytes is also high, so getting nutrients and removing waste products are necessary. So, vascularization of cardiac tissue is a critical factor in developing functional heart tissue and organs [Mesenchymal–endothelial transition contributes to cardiac neovascularization] [31–34]. Networks of capillaries in the native cardiac tissue help in transporting nutrients, oxygen, and wastes [31, 33, 34].

In heart-on-a-chip, integrating blood vessels to enhance perfusion of cardiac tissue has been tried by investigators [19, 35–37]. In cardiac spheroids and organoids, the conventional strategy is to include endothelial cells and allow the self-organization of endothelial cells into interconnected capillaries structures. However, self-formed capillaries were limited because of the variability and less efficiency [32]. Therefore, investigators tried transwell-mediated layering of endothelial and cardiomyocytes [36, 37]. Later, bioprinting 3D microfibrous scaffolds emerged for engineering endothelialized myocardium and heart-on-a-chip to generate volumetric cardiac tissues containing embedded endothelial networks [38]. Bioprinted sacrificial microfibers within hydrogel matrices, followed by removal of the template and the hollow microchannels, can be capillarized using endothelial cells to generate perfusable microvessels [39–42]. In addition, a hybrid approach where guided self-assembly and 3D bioprinting can be combined to develop endothelialized tissue constructs by encapsulating endothelial cells within the GelMA-alginate bioink fabricates scaffolds possessing a biomimetic anisotropic pattern [43]. These scaffolds will be cellularized using myocytes to generate highly perfused cardiac constructs. Zhao et al. designed a micro bioreactor to generate microtissues using rat cardiomyocytes and hESC [44]. These microtissues, named Biowire, were perfusable and developed using a PTFE tubing template. Electrical stimulation of the cardiomyocytes was achieved using carbon rod electrodes. Nitric oxide was perfused to the cardiomyocytes from a solution of sodium nitroprusside that diffused through the tubing to demonstrate the performance of the Biowire. This slowed

down the beating of cardiomyocytes which rightly assessed the toxic effects of nitric oxide.

18.4.5 Attaining Cardiomyocyte Maturity

The stem cell-derived cardiomyocytes need to mature to mimic the adult cardiomyocytes present in vivo. There are differences in the characteristics of stem cells-induced cardiomyocytes and that of adult cardiomyocytes. The morphology and structure of cardiac cells in adult phenotypes develop in about 10 years. These well-aligned, multinucleated cells have a rod shape with highly organized and developed sarcomeres, transverse tubules, sarcoplasmic reticulum, and intercalated disks with mature electrical and mechanical junctions. On the other hand, hiPSC-CMs are small, rounded, mononucleated cells with poorly organized sarcomeres and sarcoplasmic reticulum and have no transverse tubules. The electrophysiological properties of adult cardiomyocytes are also quite different from the stem cells-derived cardiomyocytes. For example, adult cardiomyocytes are stimulated with a force of around 40–80 mN/mm^2, while in hiPSC-CMs, this is around 0.08–4 mN/mm^2. The source of energy production can also be used to evaluate the extent of maturation of cardiomyocytes. In adult cells, the major source of energy production is the β-oxidation of fatty acids, while it is glycolysis in immature cells. Thus, mature cardiomyocytes are characterized by:

1. Expression of specific genes.
2. Composition and organization of the cytoskeleton and contractile apparatus.
3. Electrophysiological characteristics.
4. Oxidative metabolism.

The lack of maturation is a significant limitation to model stem cell-derived cardiomyocytes in the heart on a chip system, while using in vitro differentiated cardiomyocytes. Several techniques are being followed to improve the maturation of cardiomyocytes in vitro. One method is to increase the time for culture. But this is often a challenge as the adult cardiomyocytes maturation takes years together. Various biochemical signalling mechanisms are activated during CM differentiation in vivo. Thus, mimicking those pathways using substances like triiodothyronine and glucocorticoids in the culture can improve the maturation of stem cells-based cardiomyocytes. However, biochemical activation is not sufficient for the full development of cardiomyocytes. Cardiomyocytes undergo various mechanical stress during the maturation process and thus provide mechanical stimulation such as mechanical load, substrate stiffness, and patterning electric and magnetic field stimulation, and forced medium flow aids the maturation process [5, 6, 10, 45, 46]. In addition, studies also show that cardiomyocytes are more mature when co-cultured with cardiac fibroblasts and other non-myocyte cells [47]. The mechanical stress and electrical stimulation can be mimicked in vitro by exposing the stem cells-induced cardiomyocytes to continuous controlled biophysical stimulation

using different techniques. The electrophysiological responses of cardiac muscle cells have important roles in tissue development, maintenance, regulation, and regeneration. Electrodes are typically employed in heart-on-a-chip devices to measure the cardiac cells' electrical signals and stimulate them. The electrodes are patterned as microelectrode array using lithography to enhance the resolution of the signal. One method introduced carbon nanotubes or conductive silicon nanowires into the culture, then exposed the cells to electrical stimulation. So, attaining cardiomyocyte maturity to an adult phenotype in vitro is an important goal to be fulfilled in cardiomyocyte research.

18.5 Heart-on-a-Chip Technology: Cardiac Cells in a Microfluidic Environment

In cardiovascular research, significant developments are happening to bring physiologically realistic and complex human cells on organ-on-a-chip systems to overcome the limitations of 2D culture and animal models. The in vitro monolayer cultures commonly used to model cardiovascular diseases and for drug screening/sensitivity studies are 2D monolayer cultures [48]. In 2D cultures, the cells are randomly oriented with no parallel arrangement, whereas cells are arranged parallelly in in vivo tissue. In addition, the myocardial tissue in vivo is under constant mechanical and physical stimulation, which helps them maintain the parallel organized muscle fibre structure. Sheer stress and blood flow also determine the cardiac cell structure, phenotype, and alignment. Thus, monolayer cultures without mechanical and electrical stimulation and shear stress are not similar to in vivo physiological conditions. The microfluidic systems with proper electrical and mechanical stimulation with flow shear stress solve the problems associated with 2D cultures mimicking in vivo heart tissue. In addition, integrating suitable electrodes and sensors allows continuous monitoring of cell behaviour. By providing continuous media perfusion and suitable extracellular matrix coating, we can mimic in vivo conditions more accurately [49, 50]. Investigators can regulate the flow rate of the culture medium and provide controlled culture parameters and defined sheer stress. Thus, heart-on-a-chip technology enables high-resolution and real-time molecular sensing and reporting of complex biosystems, gaining insights about disease mechanisms, drug sensitivity, or drug mechanisms of action. Moreover, the number of cell requirements is less and thus low reagent requirement and low power consumption, which reduce the cost of experimentation in heart-on-a-chip systems.

18.5.1 Design of Heart-on-a-Chip

The heart-on-a-chip should be able to mimic the cardiac environment closely. A typical heart-on-a-chip device contains microfluidic channels for the culture of cardiomyocytes, side channels for perfusion of drugs and nutrients necessary for the growth of cells, electrodes for the stimulation of cardiomyocytes into mature

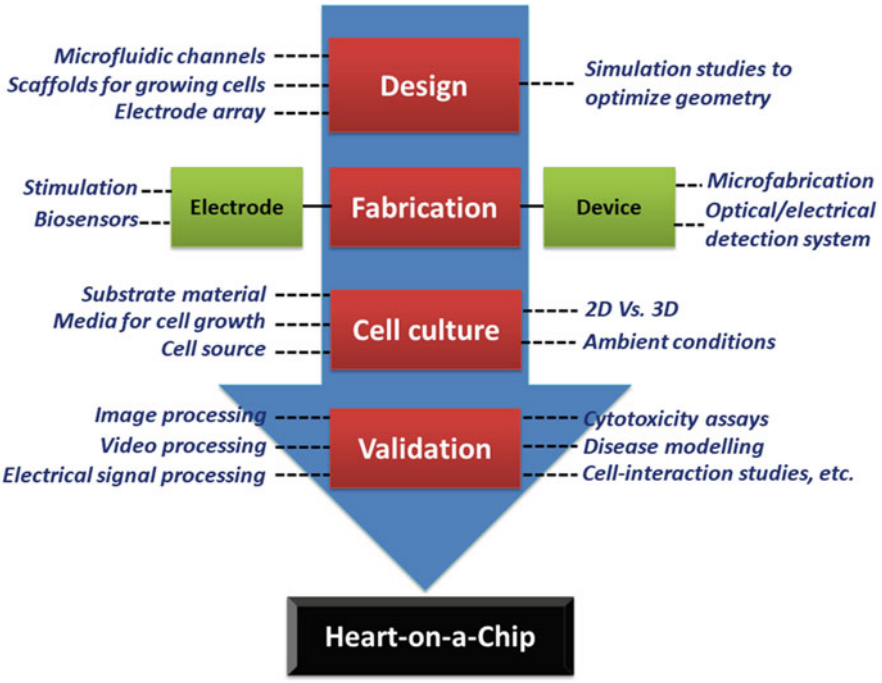

Fig. 18.3 Schematic illustration representing the main components for the development of a heart-on-a-chip

cells, and electrode arrays for measuring and recording the electrophysiological performance of the cultured cells. A schematic representation of the development of a heart-on-a-chip is shown in Fig. 18.3.

The main factors that must be considered for a heart-on-a-chip are cell source, flow dynamics, and the design of the chip are as follows:

- Cell source
  The cell source for culturing cardiomyocytes can be obtained from animals or human embryonic/fetal/adult stem cells. The most reliable cell source for growing cells in the microfluidic chips is induced pluripotent stem cells (iPSC). The fibroblasts taken from human skin are converted into stem cells by following a set of standard protocols. The stem cells are provided with chemical and mechanical signals that guide them to differentiate and grow into desired cell types.
- Flow dynamics
  Microfluidics offer an excellent capability to control flow dynamics essential for developing heart-on-a-chip devices, including nutrient and oxygen flow rates. For example, the fluid flow velocity to the chip can be adjusted using a microsyringe pump. Also, the microchannel dimensions can be modified to induce mechanical stress on the cells that can mimic the physiological conditions of the cells in vivo.

- Design
  A heart-on-a-chip platform usually consists of microchannels and microchambers patterned on a polymeric substrate and sealed with another polymer layer or a glass slide. The microchannels are often surface-treated to enhance cell adhesion to the substrate. The cardiomyocytes are cultured on the chip and are nourished with nutrients that are usually provided through side channels. The simplest design consists of 2D compartments where the cells are cultured on the main chamber and nutrients are perfused from the side channels to the main channel. The side channels also serve to provide biochemical stimulation and transportation of electrical signals. Microposts or nanofibrous meshes are also sometimes included to provide a scaffold for the 3D culture of heart cells. The growth of the cells is monitored using sensors and imaging devices. The cells are stimulated to mature using microelectrode arrays or interdigitated electrodes.

18.5.2 Simulation Studies for Heart-on-a-Chip

Simulation studies have been done to optimize the geometry of several components of a heart-on-a-chip device to enhance efficiency. These studies can predict the performance of functional components before the actual fabrication and may lead to saving time and costs. Marsano et al. had performed simulation studies to identify the uniaxial cyclic strain of the heart cells when the substrate poly(dimethylsiloxane) (PDMS) membrane is loaded with gels in response to a pressure waveform [51]. The geometric model consisted of a PDMS membrane, PDMS post, gels, and a periodic cell that could represent the entire cell culture, and owing to its symmetry, half of the cell was only modelled. It was seen from their simulation results that the majority of the bulk of the gels elongated uniaxially in response to an applied elongation. Oyunbaatar et al. had employed finite element-based modelling to analyse the extent of deflection of PDMS microgrooves based on the force applied. The spring constant of the micropillar for different dimensions was analysed and optimized for microgroove fabrication [52]. Finite element methods were also used to evaluate the resonant frequency of piezoresistive cantilevers to measure the contractile force of cardiac cells [53].

18.6 Fabrication of Heart-on-a-Chip Devices

18.6.1 Fabrication of Microfluidic Channels

Most of the reported heart-on-a-chip devices are fabricated by soft lithography techniques using PDMS from SU-8 mould obtained by photolithography. Briefly, SU-8 photoresists are spin-coated on a silicon substrate and carefully patterned using UV-laser either by direct-write lithography or mask aligners. The micro-patterned SU-8 mould serves as the master, which is then used as the template for soft lithography. PDMS polymer, consisting of a base agent and a curing agent, is

thoroughly mixed and dispensed on the surface of the mould, followed by curing the polymer and peeling it from the mould. The different layers of the device thus obtained are bonded together using surface treatments like corona discharge. Other techniques, including patterning hard polymers substrates like poly (methylmethacrylate), are also followed to develop the master template/mould. These hard polymers can be patterned according to requirements using laser or by high-speed milling machines.

18.6.2 Fabrication of Electrodes

Microelectrode arrays have been widely used to monitor parallel electrical activities from multiple cells in a culture over a long time. The electrodes for the device can be obtained by patterning a metalized substrate or by lift-off techniques. For obtaining the microelectrode array by patterning a metalized substrate, the substrate, either glass or silicon, is usually sputtered with gold, chromium, or titanium. This is followed by spin-coating a layer of photoresist on the substrate. After pre-baking the substrate, the photoresist is patterned using photolithography by selectively exposing the substrate to UV-laser. This leads to a patterned photoresist layer on the metalized substrate, where selective areas are masked with the photoresist, and the other regions are exposed to the metalized substrate. This is followed by etching the exposed metal and photoresist removal to obtain the microelectrode array. In the lift-off technique, the substrate is spin-coated with a photoresist followed by patterning the photoresist by exposing it to UV-laser. This leads to a substrate with the selective areas having the micro-patterned photoresist and other areas exposed to the base substrate. The entire substrate is then coated with a metallayer, followed by lift-off of the photoresist. This leads to the removal of the photoresist along with the metal deposited on it. Therefore, patterned microelectrode arrays of the metal are obtained on the bare substrate. These microelectrodes are commercially available and can also be directly obtained for experiments. Electrical stimulation of the cells is also achieved by inserting electrodes (platinum wires or carbon rods) into the device, which are then connected to an external stimulation source. The microelectrode array is modified with nanomaterials like graphene to improve the signal-to-noise ratio of the conventional 2D planar electrodes [54].

Although conventional 2D microelectrode arrays are useful for monitoring the extracellular action potential of the cardiomyocytes, they are limited due to their reduced signal strength and quality. 3D electrodes have been designed and developed to improve the signal quality (Fig. 18.4). Cools et al. reported the development of a microelectrode array with 3D carbon nanotubes [55]. A microelectrode array was fabricated using the lift-off technique followed by electron beam evaporation of carbon nanotube catalysts on the microelectrode array. The substrate was then transferred to a CVD chamber for CNT growth. The tangled top layer of the CNT was removed by plasma etching. The substrate was then introduced to acetone vapours to induce capillary interactions and form the final thick layer of 3D carbon nanotubes. This well-aligned 3D CNT electrode could entrap the cells and increase

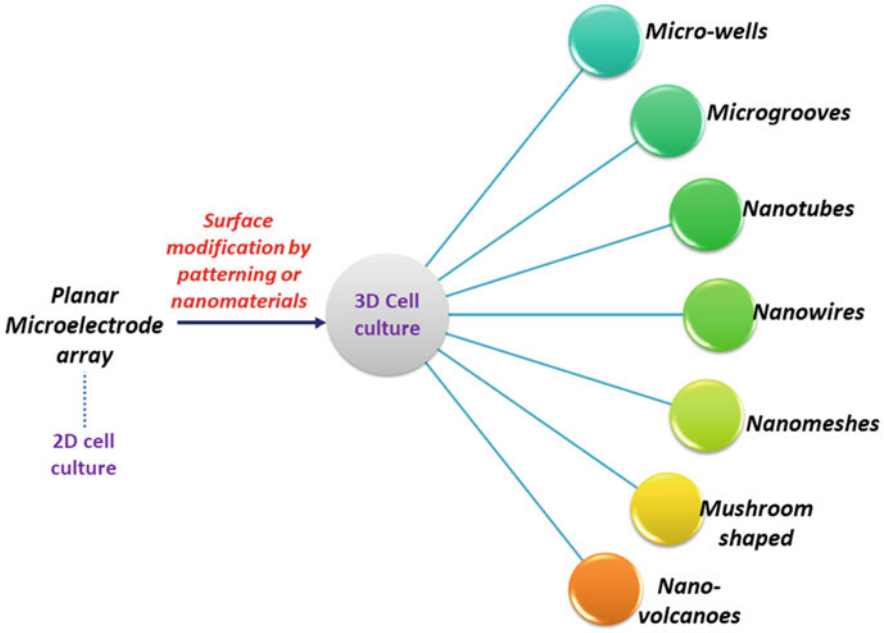

Fig. 18.4 2D cell culture to 3D cell culture: Improving the performance of heart-on-a-chip

the surface area of the electrode-cell interface. A mushroom-shaped 3D electrode array was developed by Hai et al. [56]. The fabrication process consisted of metalizing a silicon/glass substrate and then spin-coating a photoresist layer on the metalized substrate. The substrate is then patterned by photolithography to produce holes through the photoresist. This leads to the metalized substrate with a photoresist layer having holes on them to expose the metal on the substrate through the holes. Gold electroplating is then performed on the exposed metals to form artificial gold spines. After the formation of gold spines, the photoresist is removed, thereby forming a 3D mushroom-shaped array of gold electrodes.

Flexible electrodes have been developed to improve the quality of cell-substrate interaction by eliminating mechanical mismatches due to rigid substrates. A 3D self-rolled biosensor array containing a microelectrode array was developed by Kalmykov et al. [57]. The self-rolling activity of the electrodes was achieved by using a germanium sacrificial layer with polymeric support and metal electrode lines. The electrode consists of patterned chromium, palladium, and gold layers, with the germanium sacrificial layer underneath. This self-rolling 3D biosensor was successfully used to monitor the electrical activity of cardiomyocytes. A soft micropillar electrode system that can match Young's modulus of the cells in vivo was developed by Liu et al. [58]. The 3D micropillar electrode was made of conductive hydrogels developed from poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) with a conductive ionic liquid. A glass substrate was patterned with platinum interconnects using photolithography. This was passivated with SiO_2 and treated

with 2,3-dihydrothieno(3,4-b)(1,4)dioxine-2-carboxylic acid to improve the adhesion of hydrogel on platinum. Then, a mixture of PEDOT:PSS and ionic liquid was drop cast and micro-patterned with electron beam lithography followed by dry etching with an Au hard mask. The ionic liquid was finally removed in an aqueous solution to obtain the hydrogel micropillar microelectrode array. This array was connected to a printed circuit board for electrical connections and a plastic chip for cell culturing. Electrophysiological signals from the cardiomyocytes were then recorded, and it was seen that higher amplitude signals could be obtained when compared to a rigid 3D electrode. The contraction and relaxation movement of the cardiomyocytes could be mimicked due to the softness of the electrode, thereby improving the stability of the measurement.

18.6.3 Culturing Cardiac Cells

Heart cells can be engineered on different substrates using scaffolds or without scaffolds. The scaffold-free method is mainly based on two concepts. The first concept is cell-sheet engineering, where cells are generated as layers that can then be stacked to form multiple-layer structures. The cells are cultured on temperature-dependent polymers and are released from the substrate by adjusting the temperature. The second concept is the generation of spheroids of embryonic stem cells. These cells are seeded in microwells patterned on different substrates that enable the growth of these cells into different cell types, including cardiomyocytes.

In the scaffold-based method, natural or synthetic artificial matrices are used to culture the cardiomyocytes. Some natural polymers used to build the scaffold include collagen, elastin, fibrin, gelatin, alginate, and hyaluronic acid. Physical and chemical modifications are done to these polymers to improve their stability. Synthetic polymers for the scaffolds include biodegradable polycaprolactone (PCL), poly(glycolic acid) (PGA), and poly(lactic acid) (PLA). The properties of these polymers can easily be tuned according to the application. The culture of heart cells in a microfluidic environment should reflect the in vivo conditions of the heart cells rather than random cell growth in the microchannels. Therefore, the cultured heart cells in the microfluidic device should closely mimic the natural physiology of the cardiac environment. Most of the microfluidic heart-on-a-chip devices are fabricated using PDMS because of several advantages like its biocompatibility, easy handling, permeability to oxygen, non-toxicity, and low cost. Also, PDMS is elastomeric and transparent that is highly suited for cellular studies and can be rapidly prototyped into customized devices. The hydrophobic nature of PDMS is overcome by surface treatments, including corona discharge and coating with bovine serum albumin and extracellular matrix proteins like collagen, fibronectin, or laminin. Additionally, PDMS, hydrogels, or polyacrylamide (PAA) are often used to create stiff substrates that can lead to better cell maturation. Oyunbaatar et al. had developed a PDMS micropillar array device with microgrooves for creating aligned cardiomyocytes [52]. The displacement of the micropillar array due to contraction of cardiomyocytes was used to analyze the generated mechanical force. A MATLAB-

based program was used to evaluate the displacement of a large number of micropillars simultaneously. In order to overcome the drawbacks associated with the hydrophobicity of PDMS, other materials like polysulfone, polystyrene, and titanium wires have been used to culture cardiomyocytes.

Recently, bioprinting using bioinks has shown the potential to develop well-aligned cardiomyocytes that can mimic physiological conditions. This method has several advantages like easy production of customized constructs, good reproducibility, and reasonable geometry control. Hydrogels and decellularized ECM are commonly used as bioinks for bioprinting cardiac cells. Liu et al. had developed a process for the micro-continuous optical printing for bioprinting human embryonic stem cells into a hydrogel matrix on a glass substrate [59].

18.7 Characterization of the Heart-on-a-Chip Device

Electrochemical and optical methods can be used to characterize the cultured cardiomyocytes. A three-electrode system can be used to monitor the growth of cells in the device. The working electrode contains the cultured cells connected to an Ag/AgCl reference electrode and a platinum counter electrode for impedance studies. Optical evaluation of the cells can be achieved by imaging the cells using microscopes with electrical readout systems. Videos demonstrating the cellular activity and beating velocity can be obtained using electron-multiplying CCD cameras. Custom-made MATLAB codes can be used for image analysis and statistical treatments. Immunostaining and fluorescent kits can be used to monitor specific interactions of the cells. An incubator box is usually placed around the measurement system to provide the necessary conditions for the cells.

18.8 Sensing

A heart-on-chip device measures the contractility and intracellular and extracellular action potentials of the cultured cardiomyocytes. Contractility measurements provide vital information pertaining to the functioning of cardiomyocytes that can be obtained by recording the contraction of beating cells by monitoring force, frequency, and synchronizations of the cardiac contractions. Action potential measurements of cardiomyocytes are necessary for distinguishing normal cells from malfunctioning cells. These measurements are performed by optical or electrical methods. Some of the reported methods are discussed below.

18.8.1 Optical Methods

In this technique, light-based signals are used to visualize the contractility of the heart directly. Optical monitoring of cardiac cells is achieved using fluorescent probes and includes techniques like video analysis, Traction Force Microscopy

(TFM), Atomic Force Microscopy (AFM), calcium imaging, laser, and colourimetric sensing. A light source (xenon, halogen or mercury lamps, or light-emitting diodes) is used to illuminate the cells and photodetectors monitor the signal changes. The different photodetectors used for monitoring contractility include photomultiplier tubes, photodiode arrays, charge-coupled devices, and complementary metal-oxide semiconductor cameras. Potentiometric dyes, whose fluorescent properties change in response to cell membrane potentials, are used to monitor cardiac action potentials of cardiac cells (e.g. styryl dyes, Pittsburgh I to IV and VI to VIII). Calcium-sensitive dyes are used to record calcium ion transients in cultured cardiomyocytes (e.g. Fura-4F dyes, and genetically encoded calcium ions indicator proteins) [60].

18.8.1.1 Contractility Measurement

Beussman et al. had demonstrated a micropost-based technique to measure the contractile forces of cardiomyocytes where the micropost functioned as cantilever beams [61]. Cardiomyocytes were adhered to the tip of the microposts using extracellular matrix protein. Videos were captured to obtain live imaging, and image analysis code was used to quantify the deflections of the microposts. Oyunbaatar et al. had explained a procedure for analysing the contractile force generated by cardiomyocyte cultures on microgrooves on top of micropillars of PDMS [52]. An inverted microscope with 25 frames per second was used to capture the mechanics of the cultured cells on the microgrooves. ImageJ software was used to process the obtained data. The displacement of each of the micropillar could be obtained by analysing different frames. Schubert et al. had devised a method to monitor the contractile movement of cardiomyocytes using biointegrated lasers arranged in a whispering gallery mode pattern to obtain measurements with single-cell resolution [62]. The change in emission and spectral properties of the laser in response to the refractive index was used to track individual cells and assess their contractility.

18.8.1.2 Measurement of Action Potential

Pioner et al. had developed an optical setup to measure action potential and calcium ion transients of cultured hiPSC-cardiomyocytes using fluorescent indicators [63]. The action potential was recorded at the end of 75 days and 90 days using voltage-sensitive dye, FluoVolt, and calcium transients were recorded using indicator Cal630 at frequencies of 1 and 2 Hz. By simultaneously measuring these parameters with single-cell resolution, the mechanisms of cardiac contractility were evaluated. Dipalo et al. had measured the intracellular action potential of cultured cardiomyocytes using pulsed laser irradiations on graphene microelectrodes [64]. Fuzzy 3D graphene was grown on microelectrodes, and cardiomyocytes were cultured on them. The cell membrane of the cultured cells was optoporated using laser pulses, thereby providing direct contact between the intracellular matrix and the graphene electrode. Melle et al. used optoacoustic poration on cardiomyocytes cultured on commercially available microelectrode arrays (both porous and non-porous) to record the intracellular action potential [65]. Aneodymium:yttrium–aluminum–garnet (Nd:YAG) solid-state laser with a repetition rate of 80 MHz and a

pulse width of 8 ps was used for the poration of cell membranes. An upright microscope was used for the recordings, and a 60× immersion-mode objective was used to focus the laser. High-quality cardiac action potentials could be recorded using this technique.

18.8.2 Electrical Methods

18.8.2.1 Contractility Measurement

The electrical methods for monitoring contractility include impedance sensors, crack-based sensors, and strain sensors. Impedance sensors measure the changes in impedance of sensors due to the contractile function of the cardiac cells which alters the space between the cells, and the electrode is used to measure the growth, proliferation, and contractility of the heart. In strain sensors, the contraction of the cardiac cells results in changes in the electrical signal, like resistance, which is recorded. Matsudaira et al. had reported the development of piezoresistive-based cantilevers for determining the contractility forces of cardiomyocytes with a resolution of up to 0.1 nN [53]. A wheat-stone bridge circuit along with an instrumentation amplifier was used to evaluate the contractile forces. Crack sensors offer high flexibility, durability, and displacement sensitivity. Kim et al. had reported the development of a crack sensor with a cantilever made of silicone rubber for measuring the contractile force of cardiac cells [66]. The sensor could retain the gauge factor for up to 26 days and could demonstrate the toxic effects of drugs on cultured cardiac cells.

18.8.2.2 Measurement of Action Potential

Desbiolles et al. had reported a method to overcome the limitation associated with cell membrane poration for intracellular measurements [67]. They had developed a nano-patterned microelectrode array having a volcano shape that could fuse with the cell membrane of the cardiac cells, thus providing access to the intracellular matrix. The nano-patterned electrode array was fabricated by photolithography and ion-beam etching, followed by modifications with alkanethiols that could fuse with the cell membrane. Signals obtained from cultured neonatal rat cardiomyocytes confirmed the successful intracellular access of the developed nano-volcanoes. Hu et al. reported the fabrication of a microelectrode-interdigitated array to record mechanical and electrical signals from cultured cardiomyocytes [68]. Electrochemical impedance spectroscopy was used for the electrochemical evaluation of the cultured cardiomyocytes, where the mechanical beating and extracellular action potentials could be recorded. Qin et al. had developed a cardiac platform for the simultaneous non-invasive monitoring of cardiac cell growth, contractility, and electrophysiological parameters [69]. The platform consisted of microelectrode arrays connected to a multi-channel recording system for recording the potentials and interdigitated electrodes for performing impedance measurements to evaluate cell growth and contractility. The size of the microelectrodes should be comparable to cell size for the high-resolution recording of the action potentials of individual

cells. The performance of the cardiac platform was validated using blebbistatin and norepinephrine drugs. Fang et al. had reported the development of an electrical-mechanical synchronized system for measuring the mechanical beating and field potentials of the cultured cardiomyocytes [70]. The platform consists of 8 independent units, each consisting of microelectrode arrays, interdigitated electrodes, and reference electrodes. The interdigitated electrodes performed electrochemical impedance spectroscopy to monitor the beating frequency, while the microelectrode array could monitor the electrical signals from the cultured cardiomyocytes. The performance of the platform was validated by monitoring the response of the cardiomyocytes to the drugs, lidocaine and isradipine.

18.9 Applications of Heart-on-a-Chip

18.9.1 Modelling Cardiac Diseases Using Heart-on-a-Chip

Modelling cardiac diseases using human cells in heart-on-a-chip helps understand the disease mechanisms at molecular and physiological levels and develop better therapeutic options. For example, cardiomyocytes have high oxygen demand, and sudden disruption of blood supply to cardiac tissue results in hypoxia. In myocardial infarction or ischemia, hypoxia happens because of the blockage in a blood vessel-carrying oxygen to cardiac tissue. Ren et al. designed a heart-on-a-chip device with well-controlled oxygen concentration to study the hypoxia-induced changes in the myocardium [71]. An increase and decrease in Ca^{2+} concentration determine cardiac muscle contraction. Using a heart-on-a-chip, investigators found the hypoxia would induce the reversible change of Ca^{2+} concentration in cardiomyocytes [72]. Similarly, Liu et al. made a non-uniform oxygen distribution model to mimic the blockage of coronary arteries and the downstream electrophysiological changes in response to myocardial hypoxia [73].

Cardiac injury leads to the loss of cardiomyocytes. In the adult, the lost myocytes are replaced with non-functional fibrotic tissue. Fibrotic tissue can exert a mechanical load in the cardiac tissue. Also, it can affect the electrical conductivity and thus can result in irregularities in cardiac rhythm, leading to arrhythmia and heart failure. Heart-on-a-chip can model cardiac fibrosis by having appropriate cytokines to induce fibrosis, controlling the number of fibroblasts and the concentration of extracellular matrix in the microenvironment.

Studies show that increasing the fibroblast density can reduce the contraction force [74]. The contractile force and displacement of cardiac tissue are measured in cardiac tissue using traction force microscopy [75]. In a HOC model of cardiac fibrosis with iPSC-derived cardiomyocytes and cardiac fibroblasts, the authors measured the contractile force of cardiac tissue undergoing fibrosis. The pulsating strength was measured by inserting PDMS rods. The deflection of the rod because of the contraction of the cardiac tissue was measured. In another fibrotic model where cardiac fibroblasts with cytokines were embedded in gelatinmethacryloyl (GelMA) hydrogels, cyclic gradient compressions and contractile forces were measured as the

readout of fibrosis [76]. In addition, live-cell imaging and gene expression analysis were also possible on the cardiac tissue [77].

Several studies used 3D hydrogel microtissues to model cardiac fibrosis where the microenvironment is close to the native tissues, and we can control the mechanical properties [78]. Thus, the fibrotic model of cardiac tissue can be used to study the molecular level pathophysiology of cardiac fibrosis and thus expect to produce more effective antifibrotic therapies. Cardiac arrhythmia is the abnormal heartbeat rhythm because of impaired cardiac electrical activity in the heart [79]. The heart-on-a-chip 3D in vitro arrhythmia model used iPSCs-derived cardiomyocytes and filamentous matrix to fabricate the 3D microtissues. In the model, the authors measured the electrophysiological signals and contraction force related to arrhythmia and the drug response to arrhythmia [80]. Investigators modelled genetic diseases such as mitochondrial cardiomyopathy, Barth syndrome, and PRKAG2 cardiomyopathy in heart on-chip systems [80, 81]. In addition, 3D heart tissue can reveal beating changes that were not visible in 2D cultured cells. For example, cardiac microtissues derived from titin (TTN) variant-carrying iPSC cardiomyocytes beating against flexible silicone posts revealed the beating defects, while the same cells in 2D culture did not detect [82]. Similarly, it is possible to model heart failure by mimicking mechanical overload in cardiac tissue in heart-on-a-chip. In addition, it is possible to interrogate how fluid flow, cell-cell interactions, and other mechanical and electrical cues that contribute to cardiac disease development in heart on-chip models.

18.9.2 Drug Sensitivity Testing

After preclinical studies in animal models, most of the new therapeutic agents finally fail in clinical studies, leading to the withdrawal of the drugs. Moreover, the anticancer drugs in the market or clinical studies show adverse cardiotoxic effects, making them no longer helpful for chemotherapy. Heart is made up of heterogeneous cells and are present in a 3D structure, and thus drug sensitivity testing in 2D culture system may not give results that will be true in human patients. Heart-on-a-chip allows testing drugs in human cardiac cells. With the advancement in human-induced pluripotent stem cell research, scientists can generate patient-specific differentiated cardiac cells that are available to use in heart-on-a-chip. Thus, combining stem cell biology and microengineering technology will produce excellent human cardiac tissue models to test drug sensitivity to cardiac tissue and for drug development against different cardiac diseases.

18.9.3 Microfluidics for Heart-on-a-Chip Research

A 2D phenotypic in vitro model that integrated cellular systems with microelectromechanical systems was developed by Stancescu et al. to model electrophysiological and contractile force of heart cells derived from hESC-CMs [83]. A fibronectin-coated multielectrode array (MEA) was used to analyze the electrical

response of the cells, and a cantilever coated with fibronectin was used to analyze the contractile force. Photolithography technique was used to pattern the MEA for cell-adherent and cell-repellent areas. The cantilever was fabricated on a silicon wafer by photolithography and deep reactive ion etching technique. The cardiac output was monitored by measuring the electrical conductivity and contractile force generation by the cardiomyocytes. The model was used to study the toxic effects of drugs like sotalol, norepinephrine, and verapamil.

A heart-on-a-chip platform that could imitate the physiological and mechanical environment of the heart cells was developed by Marsano et al. [51]. The PDMS-based microfluidic device could produce mature cells and functional micro-engineered cardiac tissues that could mimic the mechanical movement of the heart cells in its environment. 3D constructs of the cardiac cells were developed by culturing either rat cardiac cells or human iPSC-derived cardiomyocytes in a fibrin-based gel matrix. This was polymerized using thrombin and introduced to the microfluidic platform, followed by incubation for 10 min. Auxiliary channels were also included to perfuse the culture medium for the heart cells and aided biochemical stimulation and signal transduction. The device had a caging structure consisting of hanging posts with a cell-laden solution. Gaps of fixed-width were maintained under the array of posts to define the stroke length of the cells. These hanging arrays of cells were subjected to cyclic pressure forms corresponding to the systolic and diastolic phases of heart cells for 5 days. Cell viability studies were carried out to confirm the activity of the cells, and the beating of the cells was monitored using high-resolution videos. Drug-concentration response on the developed 3D heart constructs was also performed using different concentrations of isoprenaline.

Abulaiti et al. had developed a heart-on-a-chip microdevice as a tool for pharmacological studies on heart cells [84]. The device consisted of a dynamic 3D culture of human iPSC-derived cardiomyocytes that could visualize the pulsations of cardiac cells and monitor physiological parameters like pressure, force, and fluidic output. The PDMS-based microfluidic device consisted of microchannels, chamber, diaphragm, and push bar. The microchannels were coated with fibronectin in PBS to enhance the adherence of heart cells. The 3D culture of heart cells was then transferred to the microfluidic device, and the beating of the cells was confirmed by the movement of the push bar and diaphragm. Electrical stimulations were also given to the heart cells, and the stroke volume was evaluated. A correlation was obtained for the particle displacement and beating of the culture heart cells in the frequency range of 1–2 Hz, representing the physiological situation of the heart at rest conditions. The force-frequency relationship was obtained from this, which provides vital information regarding tissue maturation and its function. The application of the device for monitoring the contractile function of the heart cells was successfully demonstrated. The dependence of intracellular calcium ions concentration on the heart cells was also confirmed. Finally, the device was tested with isoproterenol, which is a β-adrenoceptor agonist. Depending on the concentration of the drug, the beating rate of the heart varied.

A plastic chip that can be used as a scalable platform for the culture of cardiomyocytes was developed by Zhao et al. [85]. The chip could monitor passive tension, contractile dynamics, Ca^{2+} transients, conduction velocity, and action potentials. They could also engineer distinct atrial and ventricular tissues and the response of these tissues to drugs like serotonin and ranolazine was demonstrated. This platform was named Biowire and consisted of microwells patterned on polystyrene sheets and two flexible poly(octamethylene maleate (anhydride) citrate) wires glued at both the ends of the microwells array. The cardiac tissues were developed by mixing cardiomyocytes and cardiac fibroblasts with hydrogels in the microwells. The atrial and ventricular cardiomyocytes were cultured on either end of the microwells. The growth was validated using fluorescent dyes (GFP fluorescence dyes to identify atrial cardiomyocytes and MLC2v staining for ventricular cardiomyocytes). Cylindrical strips of tissues are formed in a week's time which are connected to the wires but suspended in the microwells. This platform could overcome the hydrophobic interaction of drugs observed while using PDMS substrates.

18.10 Challenges and Future Scope

Overall, there has been significant growth in developing heart-on-a-chip devices for different applications, including disease modelling and drug discovery. The heart-on-a-chip platform can be integrated with multiplexed biosensors to monitor various biomarkers that can evaluate the efficiency of the chip performance. Going forward with personalized treatments, we must develop healthy heart-on-a-chip control models with defined microenvironments and diseased heart-on-a-chip models. The disease heart-on-a-chip models are also required to develop effective treatments. Translating heart-on-a-chip models for disease modelling, drug discovery, or high-throughput cardiotoxicity screening presents various challenges. The materials used in heart-on-a-chip should be biologically compatible, inert, and without absorption and leaching properties. The heart-on-a-chip system should be robust, reliable, and reproducible. Even more, it should also be user-friendly and cost-effective. The standard material, PDMS, used in microfluidic fabrication, has absorption and leaching issues although biocompatible. Other materials such as glass or thermoplastics such as poly(methylmethacrylate), polystyrene, polycarbonate, and cyclic olefin copolymer have less absorption and leaching properties [86]. Another significant limitation in the heart-on-a-chip device development is the immaturity of iPSC-derived cardiomyocytes. iPSC-derived cardiomyocytes are more similar to fetal myocytes and constitute a significant challenge to solve.

The integration of human-induced pluripotent stem cells-based heart-on-a-chip platforms with advanced microfluidics, biosensors, and microelectronics will be highly valuable for disease diagnosis, drug discovery, personalized medicine, and therapeutics.

References

1. Cook D et al (2014) Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. Nat Rev Drug Discov 13(6):419–431. https://doi.org/10.1038/nrd4309
2. Grosberg A, Nesmith AP, Goss JA, Brigham MD, McCain ML, Parker KK (2012) Muscle on a chip: in vitro contractility assays for smooth and striated muscle. J Pharmacol Toxicol Methods. https://doi.org/10.1016/j.vascn.2012.04.001
3. Wang G et al (2014) Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. Nat Med. https://doi.org/10.1038/nm.3545
4. McCain ML, Sheehy SP, Grosberg A, Goss JA, Parker KK (2013) Recapitulating maladaptive, multiscale remodeling of failing myocardium on a chip. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1304913110
5. Nunes SS et al (2013) Biowire: A platform for maturation of human pluripotent stem cell-derived cardiomyocytes. Nat Methods. https://doi.org/10.1038/nmeth.2524
6. Abilez OJ et al (2018) Passive stretch induces structural and functional maturation of engineered heart muscle as predicted by computational modeling. Stem Cells. https://doi.org/10.1002/stem.2732
7. LaBarge W et al (2019) Maturation of three-dimensional, hiPSC-derived cardiomyocyte spheroids utilizing cyclic, uniaxial stretch and electrical stimulation. PLoS One. https://doi.org/10.1371/journal.pone.0219442
8. Zhang W et al (2017) Maturation of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) in 3D collagen matrix: Effects of niche cell supplementation and mechanical stimulation. Acta Biomater. https://doi.org/10.1016/j.actbio.2016.11.058
9. Hirt MN et al (2014) Functional improvement and maturation of rat and human engineered heart tissue by chronic electrical stimulation. J Mol Cell Cardiol. https://doi.org/10.1016/j.yjmcc.2014.05.009
10. Ronaldson-Bouchard K et al (2018) Advanced maturation of human cardiac tissue grown from pluripotent stem cells. Nature. https://doi.org/10.1038/s41586-018-0016-3
11. Parameswaran R et al (2019) Optical stimulation of cardiac cells with a polymer-supported silicon nanowire matrix. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1816428115
12. Orlova Y, Magome N, Liu L, Chen Y, Agladze K (2011) Electrospun nanofibers as a tool for architecture control in engineered cardiac tissue. Biomaterials. https://doi.org/10.1016/j.biomaterials.2011.04.042
13. Nattel S (2018) Electrical coupling between cardiomyocytes and fibroblasts: experimental testing of a challenging and important concept. Cardiovasc Res. https://doi.org/10.1093/cvr/cvy003
14. Sachse FB, Moreno AP, Abildskov JA (2008) Electrophysiological modeling of fibroblasts and their interaction with myocytes. Ann Biomed Eng. https://doi.org/10.1007/s10439-007-9405-8
15. Vasquez C, Benamer N, Morley GE (2011) The cardiac fibroblast: Functional and electrophysiological considerations in healthy and diseased hearts. J Cardiovasc Pharmacol. https://doi.org/10.1097/FJC.0b013e31820cda19
16. Maleckar MM, Greenstein JL, Giles WR, Trayanova NA (2009) Electrotonic coupling between human atrial myocytes and fibroblasts alters myocyte excitability and repolarization. Biophys J. https://doi.org/10.1016/j.bpj.2009.07.054
17. Rashedi I, Talele N, Wang XH, Hinz B, Radisic M, Keating A (2017) Collagen scaffold enhances the regenerative properties of mesenchymal stromal cells. PLoS One. https://doi.org/10.1371/journal.pone.0187348
18. Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T (1981) Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness. Science (80-). https://doi.org/10.1126/science.7008197
19. Caspi O et al (2007) Tissue engineering of vascularized cardiac muscle from human embryonic stem cells. Circ Res. https://doi.org/10.1161/01.RES.0000257776.05673.ff

20. Lesman A et al (2010) Transplantation of a tissue-engineered human vascularized cardiac muscle. Tissue Eng A. https://doi.org/10.1089/ten.tea.2009.0130
21. Iyer RK, Chiu LLY, Radisic M (2009) Microfabricated poly(ethylene glycol) templates enable rapid screening of triculture conditions for cardiac tissue engineering. J Biomed Mater Res A. https://doi.org/10.1002/jbm.a.32014
22. Iyer RK, Chiu LLY, Vunjak-Novakovic G, Radisic M (2012) Biofabrication enables efficient interrogation and optimization of sequential culture of endothelial cells, fibroblasts and cardiomyocytes for formation of vascular cords in cardiac tissue engineering. Biofabrication. https://doi.org/10.1088/1758-5082/4/3/035002
23. Naito H et al (2006) Optimizing engineered heart tissue for therapeutic applications as surrogate heart muscle. Circulation. https://doi.org/10.1161/CIRCULATIONAHA.105.001560
24. Stevens KR et al (2009) Physiological function and transplantation of scaffold-free and vascularized human cardiac muscle tissue. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.0908381106
25. Thavandiran N et al (2013) Design and formulation of functional pluripotent stem cell-derived cardiac microtissues. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1311120110
26. Zhao Y et al (2020) Engineering microenvironment for human cardiac tissue assembly in heart-on-a-chip platform. Matrix Biol. https://doi.org/10.1016/j.matbio.2019.04.001
27. Souders CA, Bowers SLK, Baudino TA (2009) Cardiac fibroblast: the renaissance cell. Circ Res. https://doi.org/10.1161/CIRCRESAHA.109.209809
28. Bourkoula A et al (2016) Roughness threshold for cell attachment and proliferation on plasma micro-nanotextured polymeric surfaces: The case of primary human skin fibroblasts and mouse immortalized 3T3 fibroblasts. J Phys D Appl Phys. https://doi.org/10.1088/0022-3727/49/30/304002
29. Jang Y, Park Y, Kim J (2020) Engineering biomaterials to guide heart cells for matured cardiac tissue. Coatings. https://doi.org/10.3390/coatings10100925
30. Martewicz S et al (2019) Transcriptomic characterization of a human in vitro model of arrhythmogenic cardiomyopathy under topological and mechanical stimuli. Ann Biomed Eng. https://doi.org/10.1007/s10439-018-02134-8
31. Rouwkema J, Rivron NC, van Blitterswijk CA (2008) Vascularization in tissue engineering. Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2008.04.009
32. Bae H et al (2012) Building vascular networks. Sci Transl Med. https://doi.org/10.1126/scitranslmed.3003688
33. Moon JJ, West JL (2008) Vascularization of engineered tissues: approaches to promote angiogenesis in biomaterials. Curr Top Med Chem. https://doi.org/10.2174/156802608783790983
34. Choi SW, Zhang Y, Macewan MR, Xia Y (2013) Neovascularization in biodegradable inverse opal scaffolds with uniform and precisely controlled pore sizes. Adv Healthc Mater. https://doi.org/10.1002/adhm.201200106
35. Sakaguchi K, Shimizu T, Okano T (2015) Construction of three-dimensional vascularized cardiac tissue with cell sheet engineering. J Control Release. https://doi.org/10.1016/j.jconrel.2014.12.016
36. Chia SL, Tay CY, Setyawati MI, Leong DT (2016) Decoupling the direct and indirect biological effects of ZnO nanoparticles using a communicative dual cell-type tissue construct. Small. https://doi.org/10.1002/smll.201502306
37. Setyawati MI, Mochalin VN, Leong DT (2016) Tuning endothelial permeability with functionalized nanodiamonds. ACS Nano. https://doi.org/10.1021/acsnano5b06487
38. Zhang YS et al (2016) Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip. Biomaterials. https://doi.org/10.1016/j.biomaterials.2016.09.003
39. Lee VK et al (2014) Creating perfused functional vascular channels using 3D bio-printing technology. Biomaterials. https://doi.org/10.1016/j.biomaterials.2014.05.083
40. Bertassoni LE et al (2014) Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. Lab Chip. https://doi.org/10.1039/c4lc00030g

41. Wu W, Deconinck A, Lewis JA (2011) Omnidirectional printing of 3D microvascular networks. Adv Mater. https://doi.org/10.1002/adma.201004625
42. Miller JS et al (2012) Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. Nat Mater. https://doi.org/10.1038/nmat3357
43. Colosi C et al (2016) Microfluidic bioprinting of heterogeneous 3D tissue constructs using low-viscosity bioink. Adv Mater. https://doi.org/10.1002/adma.201503310
44. Xiao Y et al (2014) Microfabricated perfusable cardiac biowire: a platform that mimics native cardiac bundle. Lab Chip. https://doi.org/10.1039/c3lc51123e
45. Rodriguez ML et al (2019) Substrate stiffness, cell anisotropy, and cell-cell contact contribute to enhanced structural and calcium handling properties of human embryonic stem cell-derived cardiomyocytes. ACS Biomater Sci Eng. https://doi.org/10.1021/acsbiomaterials.8b01256
46. Jacot JG, McCulloch AD, Omens JH (2008) Substrate stiffness affects the functional maturation of neonatal rat ventricular myocytes. Biophys J. https://doi.org/10.1529/biophysj.107.124545
47. Giacomelli E et al (2020) Human-iPSC-derived cardiac stromal cells enhance maturation in 3D cardiac microtissues and reveal non-cardiomyocyte contributions to heart disease. Cell Stem Cell. https://doi.org/10.1016/j.stem.2020.05.004
48. Ralphe JC, De Lange WJ (2013) 3D engineered cardiac tissue models of human heart disease: Learning more from our mice. Trends Cardiovasc Med. https://doi.org/10.1016/j.tcm.2012.08.007
49. Halldorsson S, Lucumi E, Gómez-Sjöberg R, Fleming RMT (2015) Advantages and challenges of microfluidic cell culture in polydimethylsiloxane devices. Biosens Bioelectron. https://doi.org/10.1016/j.bios.2014.07.029
50. Young EWK, Beebe DJ (2010) Fundamentals of microfluidic cell culture in controlled microenvironments. Chem Soc Rev. https://doi.org/10.1039/b909900j
51. Marsano A et al (2016) Beating heart on a chip: A novel microfluidic platform to generate functional 3D cardiac microtissues. Lab Chip. https://doi.org/10.1039/c5lc01356a
52. Oyunbaatar NE, Lee DH, Patil SJ, Kim ES, Lee DW (2016) Biomechanical characterization of cardiomyocyte using PDMS pillar with microgrooves. Sensors (Switzerland). https://doi.org/10.3390/s16081258
53. Matsudaira K, Nguyen TV, Shoji KH, Tsukagoshi T, Takahata T, Shimoyama I (2017) MEMS piezoresistive cantilever for the direct measurement of cardiomyocyte contractile force. J Micromech Microeng. https://doi.org/10.1088/1361-6439/aa8350
54. Kireev D, Seyock S, Lewen J, Maybeck V, Wolfrum B, Offenhäusser A (2017) Graphene multielectrode arrays as a versatile tool for extracellular measurements. Adv Healthc Mater. https://doi.org/10.1002/adhm.201601433
55. Cools J, Copic D, Luo Z, Callewaert G, Braeken D, De Volder M (2017) 3D microstructured carbon nanotube electrodes for trapping and recording electrogenic cells. Adv Funct Mater. https://doi.org/10.1002/adfm.201701083
56. Hai A et al (2009) Spine-shaped gold protrusions improve the adherence and electrical coupling of neurons with the surface of micro-electronic devices. J R Soc Interface. https://doi.org/10.1098/rsif.2009.0087
57. Kalmykov A et al (2019) Organ-on-e-chip: Three-dimensional self-rolled biosensor array for electrical interrogations of human electrogenic spheroids. Sci Adv. https://doi.org/10.1126/sciadv.aax0729
58. Liu Y et al (2018) Soft conductive micropillar electrode arrays for biologically relevant electrophysiological recording. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1810827115
59. Liu J et al (2019) Rapid 3D bioprinting of in vitro cardiac tissue models using human embryonic stem cell-derived cardiomyocytes. Bioprinting. https://doi.org/10.1016/j.bprint.2019.e00040
60. Herron TJ, Lee P, Jalife J (2012) Optical imaging of voltage and calcium in cardiac cells & tissues. Circ Res. https://doi.org/10.1161/CIRCRESAHA.111.247494

61. Beussman KM, Rodriguez ML, Leonard A, Taparia N, Thompson CR, Sniadecki NJ (2016) Micropost arrays for measuring stem cell-derived cardiomyocyte contractility. Methods. https://doi.org/10.1016/j.ymeth.2015.09.005
62. Schubert M et al (2020) Monitoring contractility in cardiac tissue with cellular resolution using biointegrated microlasers. Nat Photonics. https://doi.org/10.1038/s41566-020-0631-z
63. Pioner JM et al (2019) Optical investigation of action potential and calcium handling maturation of hiPSC-cardiomyocytes on biomimetic substrates. Int J Mol Sci. https://doi.org/10.3390/ijms20153799
64. Dipalo M et al (2021) Intracellular action potential recordings from cardiomyocytes by ultrafast pulsed laser irradiation of fuzzy graphene microelectrodes. Sci Adv. https://doi.org/10.1126/SCIADV.ABD5175
65. Melle G et al (2020) Intracellular recording of human cardiac action potentials on market-available multielectrode array platforms. Front Bioeng Biotechnol. https://doi.org/10.3389/fbioe.2020.00066
66. Kim DS et al (2020) Highly durable crack sensor integrated with silicone rubber cantilever for measuring cardiac contractility. Nat Commun. https://doi.org/10.1038/s41467-019-14019-y
67. Desbiolles BXE, De Coulon E, Bertsch A, Rohr S, Renaud P (2019) Intracellular recording of cardiomyocyte action potentials with nanopatterned volcano-shaped microelectrode arrays. Nano Lett. https://doi.org/10.1021/acs.nanolett.9b02209
68. Hu N et al (2018) Synchronized electromechanical integration recording of cardiomyocytes. Biosens Bioelectron. https://doi.org/10.1016/j.bios.2018.06.017
69. Qian F et al (2017) Simultaneous electrical recording of cardiac electrophysiology and contraction on chip. Lab Chip. https://doi.org/10.1039/c7lc00210f
70. Fang J et al (2021) Cardiomyocyte electrical-mechanical synchronized model for high-content, dose-quantitative and time-dependent drug assessment. Microsystems Nanoeng. https://doi.org/10.1038/s41378-021-00247-0
71. Ren L et al (2013) Investigation of hypoxia-induced myocardial injury dynamics in a tissue interface mimicking microfluidic device. Anal Chem. https://doi.org/10.1021/ac3025812
72. Martewicz S, Michielin F, Serena E, Zambon A, Mongillo M, Elvassore N (2012) Reversible alteration of calcium dynamics in cardiomyocytes during acute hypoxia transient in a microfluidic platform. Integr Biol. https://doi.org/10.1039/c1ib00087j
73. Liu H et al (2020) Heart-on-a-chip model with integrated extra- and intracellular bioelectronics for monitoring cardiac electrophysiology under acute hypoxia. Nano Lett. https://doi.org/10.1021/acs.nanolett.0c00076
74. Lind JU et al (2017) Instrumented cardiac microphysiological devices via multimaterial three-dimensional printing. Nat Mater. https://doi.org/10.1038/nmat4782
75. Pasqualini FS, Agarwal A, O'Connor BB, Liu Q, Sheehy SP, Parker KK (2018) Traction force microscopy of engineered cardiac tissues. PLoS One. https://doi.org/10.1371/journal.pone.0194706
76. Kong M et al (2019) Cardiac fibrotic remodeling on a chip with dynamic mechanical stimulation. Adv Healthc Mater. https://doi.org/10.1002/adhm.201801146
77. Mastikhina O et al (2020) Human cardiac fibrosis-on-a-chip model recapitulates disease hallmarks and can serve as a platform for drug testing. Biomaterials. https://doi.org/10.1016/j.biomaterials.2019.119741
78. Sadeghi AH et al (2017) Engineered 3D Cardiac fibrotic tissue to study fibrotic remodeling. Adv Healthc Mater. https://doi.org/10.1002/adhm.201601434
79. Tse G (2016) Mechanisms of cardiac arrhythmias. J Arrhythmia. https://doi.org/10.1016/j.joa.2015.11.003
80. Ma Z et al (2014) Three-dimensional filamentous human diseased cardiac tissue model. Biomaterials. https://doi.org/10.1016/j.biomaterials.2013.10.052
81. Travis Hinson J et al (2016) Integrative analysis of PRKAG2 cardiomyopathy iPS and microtissue models identifies AMPK as a regulator of metabolism, survival, and fibrosis. Cell Rep. https://doi.org/10.1016/j.celrep.2016.11.066

82. Hinson JT et al (2015) Titin mutations in iPS cells define sarcomere insufficiency as a cause of dilated cardiomyopathy. Science. https://doi.org/10.1126/science.aaa5458
83. Stanescu M et al (2015) A phenotypic in vitro model for the main determinants of human whole heart function. Biomaterials 60:20–30. https://doi.org/10.1016/J.BIOMATERIALS.2015.04.035
84. Abulaiti M et al (2020) Establishment of a heart-on-a-chip microdevice based on human iPS cells for the evaluation of human heart tissue function. Sci Rep. https://doi.org/10.1038/s41598-020-76062-w
85. Zhao Y et al (2019) A platform for generation of chamber-specific cardiac tissues and disease modeling. Cell. https://doi.org/10.1016/j.cell.2018.11.042
86. Van Midwoud PM, Janse A, Merema MT, Groothuis GMM, Verpoorte E (2012) Comparison of biocompatibility and adsorption properties of different plastics for advanced microfluidic cell and tissue culture models. Anal Chem. https://doi.org/10.1021/ac300771z

Kidney-on-a-Chip

19

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Abstract

The Kidney-on-a-chip field is proving to be exponential helpful technology with the ability to revolutionize disease diagnosis, drug toxicology, and novel drug discovery. For the advancement of kidney-on-a-chip models, there is a prime requirement to do rigorous validation. It could be determined whether kidney-on-a-chip models can predict clinical outcomes before the severe stage and represent human-relevant physiology. Recent advances in kidney-on-a-chip models in the past decade could be screened and utilized for drug screening to evaluate the applicabilities of new therapies and models to understand disease mechanisms better. The efficiency of kidney-on-a-chip models tends to envisage that successful application in the engineering of glomerulus-on-a-chip model can provide a blueprint for novel personalized organ chips and lead to 'body-on-a-chip' models in the future. This book chapter attempts to showcase the advancement of the kidney-on-a-chip model to mimic the kidney's in vivo functioning and understand the cellular and molecular pathways in the kidney.

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19.1 Introduction

19.1.1 Kidney: Structure and Function

A kidney is a reddish-brown bean-shaped organ present in vertebrates. These bean-shaped organs regulate the stability of fluids in the body and eradicate waste products by monitoring the reabsorption, filtration, and secretion mechanisms via the nephron (a functional kidney unit). The nephron is a crucial part of the kidney as it separates ions, small molecules, and water from the blood through ultrafiltration using the sequential working of several components. The renal corpuscle is the primary filtering component of a nephron. It comprises the Bowman's space, consisting of glomerulus and renal tubules (distal convoluted and proximal tubule), which resorb the left-out molecules present in the bloodstreams [1]. The prime role of the proximal tubule is to reabsorb the important salt present in the bloodstream, such as sodium chloride and sodium bicarbonate, reversibly, and finalize the glucose reabsorption. The proximal tubule is also an individual transportation site for amino acids and significant anions (phosphate and citrates). The kidney is a key site of drug clearance [2] gluconeogenesis [3] and has considerable involvement in vitamin D metabolism [4].

Furthermore, distal convoluted tubule also has a vital role to play in sodium (Na), potassium (K), and divalent cations homeostasis, reabsorption of 5–10% of pure sodium and chloride, contribution in the secretion of potassium ions, and maintenance of systematic calcium and magnesium homeostasis [5]. On average, 180 L of filtrates pass from the renal tubule lumen per day, highlighting the role of revealing fluid shear stresses (FSS) to expose the renal apical cells (accountable for the polarization of renal cells) [6]. The estimated range of FSS in humans is 0.7–1.2 dyn/cm^2 [7]. Proximal tubule epithelial cells (PTECs) can sense the flow with sensory organelle (primary cilium) and transfer it to the cells to restructure the cytoskeleton and junctional complex and upsurge apical endocytosis [7].

The methods going on in the tubular system (efflux, influx, and intracellular metabolism) are the reasons behind the formation of drug-induced kidney illnesses [8]. The influxing of the solutes takes place at the apical and basal membranes of the PTECs, mediated via solute carrier transporters. These transporters monitor the uptake of endogenous substrates and xenobiotics in the bloodstreams from the basolateral membranes. The solute carrier transporters include organic anion transporters (OATs) and organic cation transporters (OCTs). The intracellular accrual of internalized solutes is vetoed by an effective efflux mechanism (apical membrane to the lumen).

19.1.2 Kidney Pathology

The examination of the causes and consequences of a disease or injury is entitled as pathology. Acute and chronic kidney injuries are two categories of kidney pathologies. The frequency of both categories has amplified with time [9]. Acute kidney injuries could lead to permanent kidney injuries, resulting in the growth or deterioration of chronic kidney damage. When there is an interruption in the blood supply to the kidney or a blockage in the urinary flow in the ureter (prerenal or postrenal acute kidney injuries, respectively), acute kidney damages manifest [10]. Such problems can occur, for instance, in patients suffering from urinary stone diseases or renal hypoperfusion and could lead to impaired renal blood flow and inflammatory actions and simultaneously decline the glomerular filtration rates [11].

Additionally, injuries at distinct kidney parts, like glomerulus, tubules, etc., might also result in acute kidney damage. As the kidney is a 25% cardiac output receptor and renal tubules are linked to the blood vessels, the kidney is predominantly prone to ischemic harm. Any intrarenal or systemic circulation collapse might put forth a notable influence on renal perfusion, leading to vasoconstriction, inflammation, and even cell death [12]. Following the liver, the kidney is considered the second-most targeted organ by chemicals and drugs. Fungal toxins, chemicals, heavy metals, and pharmacotherapy could lead to nephrotoxicity, resulting in acute kidney injuries linked with enhanced morbidity and mortality. Additionally, drug-induced nephrotoxicity is believed to be the reason behind 20% of overall acute kidney injuries [13]. Antibiotics, radiocontrast agents, chemotherapeutics, and nonsteroidal anti-inflammatory compounds are a few types of drugs [14]. Based on the survey, 5–7% of hospital admissions in the United States could be attributed to acute kidney injuries [15]. The number of kidney injuries rose between 1988–2003 due to increased prescription of drugs linked with nephrotoxicity [16].

Scheming low nephrotoxic potential drugs would lower the injuries in the kidney. But, considering the existing preclinical research gears, the lethal perspective of the applicant compounds is underestimated. Drug wearing away is estimated to be only 2% from the preclinical studies. However, this level rises to 9% in clinical trials, and toxic side effects of novel medicines are found in 20% of novel medicines throughout the postmarket surveillance [17]. Kidney dysfunction enhances the jeopardy of adverse drug procedures by disturbing hepatic, intestinal, and renal drug metabolisms [18]. Therefore, understanding the nephrotoxicity mechanism is very crucial in the development of effective and safe drugs. Existing models applied in the preclinical kidney experiments are unable to predict the drug-produced kidney problems and efficiently imitate the functions and structure of the kidney. Therefore, it has become imperative to solve these limitations via predictive and more dependable in vitro models. Figure 19.1 illustrates the advancement of various in vitro models progressively with time.

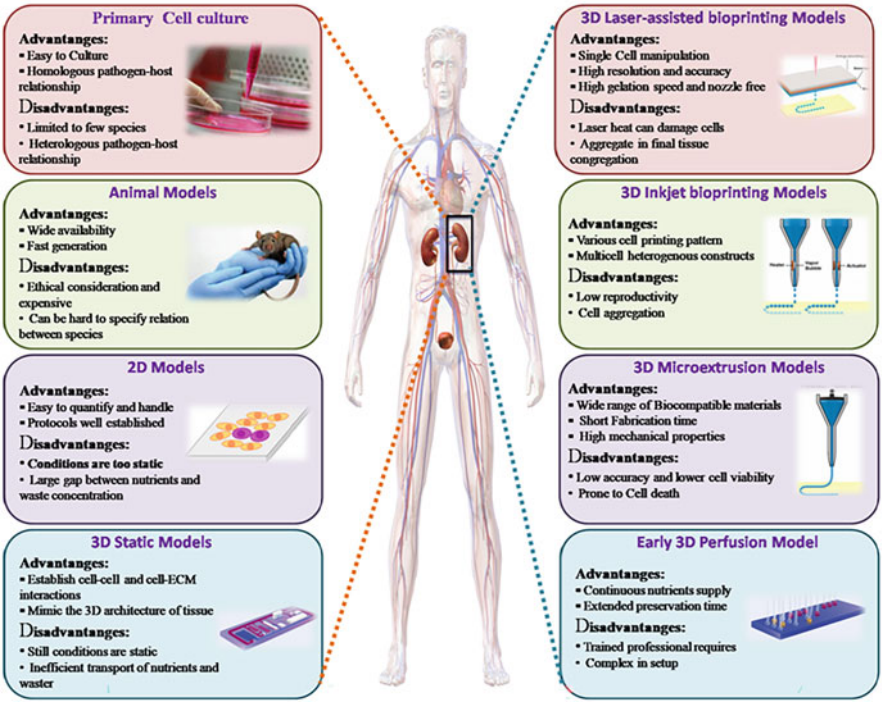

Fig. 19.1 Advancement of various in vitro models progressively with time

19.1.3 Issues Allied with the Development of In Vitro Kidney Models

A kidney comprises >10 cell types organized in a 3D arrangement with a multifaceted vascular system and enclosed via an extracellular matrix (ECM). All the characteristics must be given importance while designing a three-dimensional in vitro model that mimics renal structural and functional features. As blood and secretory circulation exhibit pathological and physiological significance in the kidney, a 3D model of the kidney must integrate a microfluidic stage to duplicate the luminal and tissue circulation. Cell culturing under a flow environment replicates the in vivo conditions and facilitates examining cell signal transduction, drug responses, and gene and protein expressions [19]. In order to fully imitate the cellular complexity, specific cell types must be cocultured to support the immune cell placement, detailed signaling routes, and most notably, cells interactions. Furthermore, it is also crucial that in vitro renal system must deliver functional transporters for apt technique justification and data understanding along with biological responses toward drugs to facilitate resemblance for in vivo responses [20]. In the tubular structures, cell performance is affected via the curved shapes of the tubules. Lastly, the chosen cell types for populating the chips must preserve the human individuality and permit the analysis of biomarkers significant to the in vivo state.

19.1.4 Kidney Models

19.1.4.1 2-D vs. 3-D Systems

In general, animal models are employed to evaluate the kidney responses; however, interspecies variances in blood circulation, speed of drug metabolization, and transporter terms prohibit extrapolating clinical factors [21]. Additionally, testing on animals raises ethical concerns. The usage of isolated ex vivo human kidney slices and tubules in drug research is hindered by their inadequate viability. The primary cells acquired from human donor bodies tend to survive only for 2 weeks and could be contaminated by transporters, and also their activity levels and expression are uncontrollable.

The involvement of 2-D cultures in in vitro research has gained much attention for a long time duration. These are useful, inexpensive, and easy to evaluate the fundamental biological processes' signal transduction and molecular mechanisms. 2D cultures have exhibited applicability for evaluating renal pathology, physiology, and pharmacology. However, they failed to replicate the renal microenvironment due to the lack of apical-basal polarization and incapability of mimicking transepithelial transport and observing the fluid shear stress, while growing in static circumstances. To design a model that would replicate kidney structures perfectly and would be appropriate for preclinical examinations, it must include 3-D spatial distribution, fluid shear stress, ECM, and compartmentalization. On comparison, 3-D models are more advanced than 2-D models as they tend to replicate the scaffolding and microenvironment of the tissues more accurately. The absence of polarization in 2D models could be attained in 3D models by using porous membranes. However, the conventional porous membrane-dependent procedures do not come up with flying colors to completely replicate renal tubular epithelial cells [22]. In 3-D cell culture, nephrotoxicity biomarkers have emerged as more reliable as compared to the 2D cultures. Moreover, a 3-D model involving kidney cortical epithelial cells was more effective than the 2-D model in evaluating drug-induced chronic carcinogenicity by finding the kidney damaging molecules and neutrophil gelatinase-linked lipocalin [23]. However, static 3D culture models are unable to achieve natural flow dynamics which is crucial for the cell functionality and polarity [24].

19.1.4.2 Cellular Models

There are multiple cellular models available for performing kidney research. PTECs are predominantly prone to drug lethal. Therefore, the advancement of in vitro models has attracted the attention of the pharmaceutical industry. Immortalized and primary cells belonging to the proximal tubules have been applied in research. Animal-gleaned cells displayed species-specific variations in the SLCOs expressions, flavin comprising monooxygenases, and the P-gp [25]. Due to the above-stated facts, the involvement of animal-gleaned cells is not recommendable. Human renal cortex immortalized cultures (for instance: human kidney 2 PTECs) are accessible [26]. Such cells preserve the phenotypes of the proximal tubule cells and functional transporters. These cells are responsive to toxicants.

Nevertheless, the immortalized cell lines require easy maintenance and are affordable. They fail to duplicate the phenotype of primary cells, are deficient in functional differentiation, and display the wrong expression of human transporters [27]. In addition, their continuous growth might lead to multilayered structures, which would further thwart the hollow fiber lumen [28]. Primary human cells are found as a better version than immortalized cells. Human PTECs are regularly employed in in vitro examination [29]. Such cells possess the functionality and expression of multiple transporters. Even though they deliver the functionality, they also tend to get infected with other cell types and preserve fewer duplication passages (highest—12 doublings) [30].

19.1.4.3 Kidney-on-a-Chip Models

The arrangement of a kidney model with a stage reproducing the structure or microenvironment of the human kidney is entitled as a kidney-on-a-chip model. Such models must permit coculturing of numerous renal cell varieties and preserve functional cell to cell interactions, endocrine and metabolic functions, and expressions for functional transporter [31]. Kidney-on-a-chip models could be engineered to guarantee a high-throughput display of drug toxicity examination to evaluate the glomerular filtration methods, enhance the drug dose estimation precision, and establish the pharmacokinetics. The demand for a model that completes the need to skip the animal dependency for preclinical testing would help predict drug-induced nephrotoxicity. Moreover, along with glomerulus, proximal, and distal tubules models, there is a tremendous requirement for the full-grown nephron-on-a-chip model to integrate components.

Although the nephron is a multicomponent system and mimicking the nephron as a functional kidney-on-a-chip is more recognizable as compared to an individual combination of components, this structural and functional arrangement along with their physiology and diffusion of various fluidic particles through native kidney interstitium is specialized in their efficiency [32]. The first mimicking interaction between the tubular cells was mannered by the Mu et al. group, forming a microtubular 3D system of composite hydrogel on both sides of primary human umbilical vein endothelial cells and MDCK renal epithelial cells have been cultured [33]. It could be genuinely righteous to formulate the first synthetically designed glomerular apparatus's working and countercurrent exchange. Moreover, the coming reports ascertain the data analysis of slower gradients, such as oxygen, chemical, and nutritional on the chip platform. Current researchers have suggested that the field of kidney-on-chip is escalating fast enough, although the complex renal physiology in vitro system may still be on the horizon.

19.2 Motivation for the Organ on the Chip Technology

The tremendous expansion of organ-on-a-chip technology is projected to be an economic driver for advanced and developing countries. It expectedly would have a global market of $6.13 billion by 2025. Moreover, the organ-on-chip technology

will give an accurate drug analysis with the integration of sensors, biophysicochemical cues, and precision control [34]. Nonetheless, the current progress will provide a convenient platform for tuning various pressure and fluid flow for the in vitro cell organization at the microliter scale [35]. The upcoming models of kidney-on-a-chip have improved the efficiency of culturing cells by providing necessary microchannels and microfluidics systems, thus mimicking the preferential tissues with multilevel functions in vitro and ex vivo.

19.3 State-of-Art Development of Multiple Kidney Components on a Chip

For the well-performed kidney physiology and pathophysiology, there will be progressive participation of all human kidney functional units in the kidney-on-chip model. Therefore, in a biomimetic kidney-on-a-chip model, integrated cell-cell interactions between podocytes and glomerular vascular endothelial cells must be in coexistence to maintain the transcellular electrochemical and osmotic pressure gradients. All these factors are necessary for a synthetic model's better efficiency of fluid flow dynamics, endocrine functions, and cellular metabolic. Despite the advancement of separated models of kidney components like proximal tubular, glomerular models, and distal tubular physiology in the synthetic domain through on-chip technology, the complete integration of all components for envisaging the true kidney-on-a-chip with single output values has yet to be formulated [36].

19.4 Kidney-on-a-Chip Physiology and Pathophysiology Models

The physiological relevance of biomimicking kidney-on-a-chip must indemnify the kidney's complexity by taking into account all critical factors of osmosis, fluid flow dynamics, endocrine functions, cellular metabolism, and integrative cell-to-cell interactions. In a recent study, the physiology and pathophysiology of thrombosis have been successfully processed with printed vascular endothelium for antibody capture and release of circulating tumor cells [37]. The kidney-on-a-chip or microphysiological system has been divided into a tubular structure, collecting duct, proximal tubule, glomerulus, and nephron depending upon the microfluidics-based studies. Until now, on-chip technology has robustly developed the physiology of glomerular, proximal, and distal tubular propitiously. Although the higher-order structures with impeccable functional efficiency have not yet been designed in vitro, new and progressive developments promise subsiding challenges of the present time.

19.4.1 Glomerulus-on-a-Chip Model

While developing the model of hypertensive glomerulopathy, Zhou et al. formulated the concept of glomerulus-on-a-chip. In this model, mouse podocyte (clone 5) precursorcells and conditionally immortalized glomerular endothelial cells are used, and layer proximity of both cells has been used to line two microfluidic channels to model hypertensive nephropathy and mimic the cellular damage, cytoskeletal rearrangements, and glomerular leakage [38]. This chip model dexterously mimics the glomerus, the kidney's main filtering unit formed by a vast network of capillaries and differentiated epithelial cells. The differentiated epithelial cells or podocytes regulate and maintain blood filtration into an ultrafiltrate to selectively remove urine [39]. Subsequently, Wang et al. developed a glomerulus-on-a-chip microdevice using rat glomerular endothelial cells to replicate the early-stage diabetic nephropathy. This model was beneficial to produce high glucose pathological responses. However, the lack of human kidney endothelial cells for developing the glomerulus-on-a-chip has been the prime limitation [40]. To overcome this limitation, terminally differentiated podocytes from human-induced pluripotent stem cells (iPSCs) have been used by Musah et al. to develop a glomerulus-on-a-chip device that mimics the molecular filtration of molecular filtration glomerular capillary walls to resemble the nephrotoxicity of in vivo [41].

Furthermore, to expand the applicability of the glomerulus-on-a-chip model and subsiding patient-specific disease modeling and personalized medicine limitations, Roye et al. engineered a personalized glomerulus chip system from human-induced pluripotent stem (iPS) cell-derived vascular endothelial cells (ECs) and podocytes from a single patient [42]. Recently, Perin and Da Sacco [43] have developed a novel glomerulus-on-a-chip model to mimic the functionality and characteristics of the glomerular filtration barrier. This model confirmed the impermeability of albumin in physiological concentrations along with free diffusion of insulin and thereby outlined the permselectivity of in vivo glomerulus.

19.4.2 Proximal Tubular-on-a-Chip

Several investigators have successfully designed the proximal tubular-on-a-chip to keep the role of the proximal renal tubule as a prime target of drug-induced toxicity and the leading site of drug clearance. Hollow fibers complete the prime requisite of producing proximal tubule in vitro, resembling the scaffold of culture renal proximal tubule cells and agility in the secretion of molecules and control reabsorption. Jang et al. seeded primary human proximal tubule epithelial cells (PTECs) on the upper surface of extracellular matrix (ECM)-coated polyester membrane to develop a proximal tubule-on-a-chip device [44]. Their work has distinctly displayed the role of cells cultured under static and in vivo fluid flow conditions, explaining the attainment of columnar shape, primary cilia, and system gained polarity by cells exposed to fluid shear stress compared to those in static conditions. Similarly, through culturing human renal progenitor cells in a microfluidic system, it was

discovered that, unlike the static conditions, the cell in fluid shear stress polarized and decreased the permeability of creatinine and urea [45]. Furthermore, seeded human PTECs preferentially on the inner surface of hollow fibers are coated with hydrogel [46]. Their work has shown the successful formation of a monolayer with functional transport capabilities for glucose transport. Jansen et al. developed a model incorporating hollow fibers mimicking the ability of functional renal tubules to exhibit secretory clearance of albumin-bound uremic toxins and reabsorption of albumin [47]. Additionally, Weber et al. developed a microfluidic device with culturing of hPTECs with tubular structures surrounding the chamber coated with ECM [48]. The excellence of this model was that it maintained >95% cell viability for up to 4 weeks of duration. With the success of the above models, microfluidic systems appeared to be perfect for mimicking physiological conditions, subsequently maintaining the PT cells in a better-differentiated state in vitro compared to conventional culture systems.

Advances in the more efficient model have provided bioprinting as an alternative technique for developing complex systems and shaping the morphology of proximal tubule-on-a-chip devices [49]. Using the bioprinting technique, Homan et al. bioprinted a tubular architecture demarcated by PTECs and have a fugitive pluronic ink on a gelatin-fibrinogen ECM [50]. Human PTECs coated with internal tubules showed improved morphology and proved to be more efficient than their 2D counterparts. Moreover, this model was stable for nearly 2 months, confirming the potential of bioprinting devices for the kidney-on-a-chip model. Consecutively, the bioprinting technique was used by King et al. to design a proximal tubule incorporating renal fibroblasts, endothelial cells, and epithelial cells. This model was stable for at least 30 days and replicated features of renal fibrosis [51]. Recently, Vormann et al. developed a 3D microfluidic platform (Nephroscreen) for the detection of drug-induced kidney injury (DIKI) in the field of pharmaceutical development [52]. Furthermore, they have investigated this model with four nephrotoxic drugs (cisplatin, cyclosporine, tenofovir, and tobramycin) and confirmed the testing with eight pharmaceutical compounds. This study proved to be a proof-of-concept study to demonstrate the usability and reproducibility of Nephroscreen for the drug-transporter interactions and detection of DIKI.

19.4.3 Distal Tubule-/Collecting Duct-on-a-Chip

In addition to the cells of the proximal tubule, few groups have tried to examine the distaltubular and cortical collecting duct-on-a-chip model for biomimicking. Similarly, Baudoin et al. developed MadinDarby canine kidney (MDCK) cells on a chip with fibronectin-coated polydimethylsiloxane (PDMS) device [53]. This study showed the role of finite fluid flow for proliferation and survival and proved that flow rates (50 μL/min) mimicking in vivo situations better-impaired cell proliferation and survival. In a similar experiment, Jang and Suh [54] maintained fluid shear stress (1 dyn/cm^2) to resemble the native renal tubules with PDMS hollow fibers coated with collagen. This work has improved cellular functionality by applying

smaller fiber diameters, highlighting the effect of surface curvature on cell functionality [54].

19.5 Clinical Applications of Kidney on Chip Models

The evaluation and integration of multiple cell types from various organs for systemic and secondary drug toxicity can be helpful for drug metabolism and detection of the drug target organ [55]. Vernetti et al. [56] tried to combine absorption, clearance, metabolism, and neurovascular with skeletal muscles models to form a combined on-chip model and showed sequential transfer of media from one organ to another [55, 56]. Li et al. also developed a model consistent with clinical digoxin nephrotoxicity [57]. In this model, they connected rat primary glomerular endothelial cells with intestine colorectal adenocarcinoma cell lines in the microchamber of the microfluidic system.

Moreover, this system efficiently evaluated the digoxin-induced glomerular endothelial toxicity and proved the manifestation of the increased permeability and cell death. The failure of one model to predict kidney injury opens a new avenue for a new trial. The failure of kidney injury, which SPC5001 ASO induces in a clinical trial, suggests the prime requisite for additional preclinical models with better capability for preceding animal toxicity studies. Target downregulation, cytotoxicity, and kidney injury biomarkers were induced by the clinically nephrotoxic ASO SPC5001, demonstrating the translational potential of this kidney on a chip. Nieskens et al. introduced nephrotoxicity and kidney injury biomarkers cultured in 2D by SPC5001 in human renal proximal tubule epithelial cells (HRPTEC) [58]. In a recent study, Cohen et al. showed the utility of kidney spheroid-on-a-chip technology imbibed with embedded sensors for monitoring cellular metabolism in response to drug treatments to formulate the mechanisms of drug-induced nephrotoxicity [59]. In the review study, Hwang et al. [60] have represented the ability of the organ-on-a-chip model to evaluate the drug efficacy in lung and kidney cancer models [60].

19.6 Challenges Towards the Kidney on the Chip Model

In the early stage, the kidney on the chip model had faced many problems. Some vasoconstrictors drugs such as calcineurin and catecholamines inhibitors cause injury to the renal medulla while reducing oxygen delivery [61]. Furthermore, the drug disturbing renal blood flow has proved to be stimulating for screening in vitro nephrotoxicity [62]. Despite all the challenges, the advancement of blood vessel-on-a-chip devices has highlighted the applicability of vasoconstrictive nature in the organ-in-chip model. Moreover, the kidney consists of 10 various types of cells arranged in a 3-D network in a complex vascular system and engrossed by extracellular matrix (ECM) [63]. In order to fully mimic the cellular complexity of the kidney through the kidney-on-a-chip model, the function of various cell types should

be formalized by specific signaling pathways, immune cell recruitment, and preferential cell-cell interactions [64]. The pathology of secretory and blood circulation plays a vital role in mimicking tissue and luminal circulation in the kidney-on-a-chip model [6]. Therefore, culture cells bearing the flow conditions created in vivo situations are helpful in the gene and protein expression [19] and study of cell signal transduction [7]. Lastly, the perfect kidney-on-a-chip model must overcome and exhibit prime kidney characteristics and enable biomarkers to showcase the relevant in vivo situation.

Linking on-chip systems to integrate sensors into fluidic systems for continuous measurement of cellular behavior has been efficient in monitoring epithelial barrier integrity and the toxicity prediction of the model [65, 66]. Moreover, the organ-on-a-chip field is progressing abruptly as the traditional animal-derived cell lines model used earlier in microfluidic systems is being replaced by iPSC-derived cells to model terminally differentiated human cells to provide helpful renal physiology. Despite the progress of the kidney-on-a-chip model, several challenges remain unresolved. One such is the limited life span of all the cells in model devices which becomes severe in primary cells. Additionally, the inadequate information of tubular drug transporters and metabolic enzymes models also hampers the kidney-on-a-chip models to predict accurate drug toxicity. Lastly, the biocompatibility of materials, certainly for clinical use, is one of the foremost challenges. Materials should be inert to biological media, bio-benign, and non-leaching nature [31]. Therefore, the integrated system of the kidney-on-a-chip model must maneuver all the functions of integrated systems while considering the pros and cons of each in vitro system for final system validation and standardization. Some of these challenges have been overcome, as the advances of stem cell technology have sided the limitations of modeling terminally differentiated cells with integrating iPSC-derived podocytes into microfluidic devices [41]. The major problem of biomimetic and dynamic tissue integration in microfluidic platforms has been overcome by developing four-dimensional bioprinting [67]. Furthermore, the precision at the molecular level of cellular diagnosis and interaction has been achieved and improved with nanotechnology.

19.7 Future Opportunities

Recently, one such challenge has been overcome by Linda et al. with tissue to urine culturing of kidney and perfused tabloids cells-on-a-chip.

F1: This is a successive step to generate a powerful in vitro tool having perfused microfluidic multi-chip platform with a combination of two essential systems with better dexterous of mimicking the complexity of kidney tubules. Additionally, the major obstacle of limited control over cellular plasticity, with which the current in vitro kidney cell models are struggling to augment cell-type-specific functionality, has been summarized to drive the maturity of kidney epithelial cells.

F2: Focusing on the kidney tissue regeneration in the kidney-on-a-chip model, the execution of cell plasticity by shifting cells to a functional state is more promising. Furthermore, the final incorporation of all supporting cells and vasculature in kidney F3 and F4 with a critical definition of microfluidic systems and functions can promulgate the synergy of nephron segments for the success of the kidney-on-a-chip model. Interestingly, the advancing nephron-on-a-chip models show promise of pathological renal physiology with predicting nephrotoxicity for the early diagnosis of chronic kidney diseases. The printing technology and advanced nanomaterials can provide significant insight into improving the kidney-on-a-chip model for prior detection of the conditions such as thrombotic microangiopathy and vascular damage. Shortly, the step-by-step improvement of various components of the kidney for the kidney-on-a-chip model enhances and evaluates the critical mechanisms of renal transplantation tolerance and rejection. Roye et al. envisaged that the ability of human iPS cells to differentiate into almost any cell type and successful application in engineering glomerulus-on-a-chip model provides a foundation for more personalized organ chip and 'body-on-a-chip' models in the future [42].

19.8 Conclusions

The summary of the above studies would be beneficial to suggest that kidney-on-chip systems might be valuable tools for predicting in vivo drug-induced proximal tubular toxicity. Although kidney-on-a-chip models are not the final solutions, they provide inevitable and preferable results compared to other in vivo processes. Recent advances in modeling vascular systems certify the current promise for vasoconstrictive nephropathy screening; thereby, multi-organ-on-a-chip models keep their promise of improving prior information of secondary toxicities. Even though kidney-on-a-chip models present new biological modeling, they still possess significant limitations which can never be circumvented; thus, alternative tools may also be tried and preferred parallelly. Furthermore, kidney-on-chip technologies for testing distal tubular drug toxicities, tubular obstruction, and interstitial nephritis remain limited. It would be right to be sure that achievements in the kidney-on-a-chip field could reveal exciting new avenues for drug discovery and development. In the coming future, there are still tremendous opportunities to scavenge the many possibilities that kidney-on-a-chip technology would solve for better physiological and pathophysiological applications.

References

1. Zhuo JL, Li XC (2013) Proximal nephron. Compr Physiol 3. https://doi.org/10.1002/cphy.c110061
2. Yeung CK, Himmelfarb J (2019) Kidneys on chips: emerging technology for preclinical drug development. Clin J Am Soc Nephrol 14. https://doi.org/10.2215/CJN.06690518

3. Meyer C, Dostou JM, Gerich JE (1999) Role of the human kidney in glucose counterregulation. Diabetes 48. https://doi.org/10.2337/diabetes.48.5.943
4. Jones G (2012) Metabolism and biomarkers of vitamin D. Scand J Clin Lab Invest 72:7–13
5. Subramanya AR, Ellison DH (2014) Distal convoluted tubule. Clin J Am Soc Nephrol 9. https://doi.org/10.2215/CJN.05920613
6. Raghavan V, Rbaibi Y, Pastor-Soler NM et al (2014) Shear stress-dependent regulation of apical endocytosis in renal proximal tubule cells mediated by primary cilia. Proc Natl Acad Sci U S A 111. https://doi.org/10.1073/pnas.1402195111
7. Essig M, Terzi F, Burtin M, Friedlander G (2001) Mechanical strains induced by tubular flow affect the phenotype of proximal tubular cells. Am J Physiol Renal Physiol 281. https://doi.org/10.1152/ajprenal.2001.281.4.f751
8. Fisel P, Renner O, Nies AT et al (2014) Solute carrier transporter and drug-related nephrotoxicity: the impact of proximal tubule cell models for preclinical research. Expert Opin Drug Metab. Toxicol 10:395–408
9. Coresh J, Selvin E, Stevens LA et al (2007) Prevalence of chronic kidney disease in the United States. J Am Med Assoc 298. https://doi.org/10.1001/jama.298.17.2038
10. Makris K, Spanou L (2016) Acute kidney injury: definition, pathophysiology and clinical phenotypes. Clin Biochem Rev 37:85–98
11. Mahmud HM, Mahmud SM (2017) Acute kidney injury in ureteric stones: single Centre short term analysis. Pak J Med Sci 33. https://doi.org/10.12669/pjms.334.13345
12. Bonventre JV, Yang L (2011) Cellular pathophysiology of ischemic acute kidney injury. J Clin Invest:121
13. Hoste EAJ, Bagshaw SM, Bellomo R et al (2015) Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. Intensive Care Med 41. https://doi.org/10.1007/s00134-015-3934-7
14. Fanos V, Cataldi L (2001) Renal transport of antibiotics and nephrotoxicity: a review. J Chemother 13:461–472
15. Lameire NH, Bagga A, Cruz D et al (2013) Acute kidney injury: an increasing global concern. Lancet 382
16. Tomlinson LA, Abel GA, Chaudhry AN et al (2013) ACE inhibitor and angiotensin receptor-II antagonist prescribing and hospital admissions with acute kidney injury: a longitudinal ecological study. PLoS One 8. https://doi.org/10.1371/journal.pone.0078465
17. Laverty HG, Benson C, Cartwright EJ et al (2011) How can we improve our understanding of cardiovascular safety liabilities to develop safer medicines? Br J Pharmacol 163:675–693
18. Dreisbach AW, Lertora JJL (2008) The effect of chronic renal failure on drug metabolism and transport. Expert Opin Drug Metab Toxicol 4:1065–1074
19. Choucha Snouber L, Jacques S, Monge M et al (2012) Transcriptomic analysis of the effect of ifosfamide on MDCK cells cultivated in microfluidic biochips. Genomics 100. https://doi.org/10.1016/j.ygeno.2012.05.001
20. Fitzgerald KA, Malhotra M, Curtin CM et al (2015) Life in 3D is never flat: 3D models to optimise drug delivery. J Control Release 215:39–54
21. Heinonen IHA, Sorop O, Merkus D, Duncker DJ (2017) Comparative physiology and pathophysiology of the coronary circulation. In: Physiological assessment of coronary stenoses and the microcirculation. Springer, New York
22. Vinaiphat A, Charngkaew K, Thongboonkerd V (2018) More complete polarization of renal tubular epithelial cells by artificial urine. Cell Death Discov 4. https://doi.org/10.1038/s41420-018-0112-z
23. DesRochers TM, Suter L, Roth A, Kaplan DL (2013) Bioengineered 3D human kidney tissue, a platform for the determination of nephrotoxicity. PLoS One 8. https://doi.org/10.1371/journal.pone.0059219
24. Jang KJ, Cho HS, Kang DH et al (2011) Fluid-shear-stress-induced translocation of aquaporin-2 and reorganization of actin cytoskeleton in renal tubular epithelial cells. Integr Biol 3. https://doi.org/10.1039/c0ib00018c

25. Hagenbuch B, Stieger B (2013) The SLCO (former SLC21) superfamily of transporters. Mol Aspects Med 34:396–412
26. Adler M, Ramm S, Hafner M et al (2016) A quantitative approach to screen for nephrotoxic compounds *in vitro*. J Am Soc Nephrol 27. https://doi.org/10.1681/ASN.2015010060
27. Jenkinson SE, Chung GW, van Loon E et al (2012) The limitations of renal epithelial cell line HK-2 as a model of drug transporter expression and function in the proximal tubule. Pflugers Arch 464. https://doi.org/10.1007/s00424-012-1163-2
28. Ozgen N, Terashima M, Aung T et al (2004) Evaluation of long-term transport ability of a bioartificial renal tubule device using LLC-PK1 cells. Nephrol Dial Transplant 19. https://doi.org/10.1093/ndt/gfh399
29. Sanechika N, Sawada K, Usui Y et al (2011) Development of bioartificial renal tubule devices with lifespan-extended human renal proximal tubular epithelial cells. Nephrol Dial Transplant 26. https://doi.org/10.1093/ndt/gfr066
30. Narayanan K, Schumacher KM, Tasnim F et al (2013) Human embryonic stem cells differentiate into functional renal proximal tubular-like cells. Kidney Int 83. https://doi.org/10.1038/ki.2012.442
31. Wilmer MJ, Ng CP, Lanz HL et al (2016) Kidney-on-a-Chip Technology for drug-induced nephrotoxicity screening. Trends Biotechnol 34:156–170
32. Huh D, Torisawa YS, Hamilton GA et al (2012) Microengineered physiological biomimicry: organs-on-chips. Lab Chip 12:2156–2164
33. Mu X, Zheng W, Xiao L et al (2013) Engineering a 3D vascular network in hydrogel for mimicking a nephron. Lab Chip 13. https://doi.org/10.1039/c3lc41342j
34. Yang Q, Lian Q, Xu F (2017) Perspective: fabrication of integrated organ-on-a-chip via bioprinting. Biomicrofluidics 11. https://doi.org/10.1063/1.4982945
35. Whitesides GM (2006) The origins and the future of microfluidics. Nature 442
36. Weinberg E, Kaazempur-Mofrad M, Borenstein J (2008) Concept and computational design for a bioartificial nephron-on-a-chip. Int J Artif Organs 31. https://doi.org/10.1177/039139880803100606
37. Zhang YS, Davoudi F, Walch P et al (2016) Bioprinted thrombosis-on-a-chip. Lab Chip 16. https://doi.org/10.1039/c6lc00380j
38. Zhou M, Zhang X, Wen X et al (2016) Development of a functional glomerulus at the organ level on a Chip to mimic hypertensive nephropathy. Sci Rep 6. https://doi.org/10.1038/srep31771
39. Greka A, Mundel P (2012) Cell biology and pathology of podocytes. Annu Rev Physiol 74. https://doi.org/10.1146/annurev-physiol-020911-153238
40. Wang L, Tao T, Su W et al (2017) A disease model of diabetic nephropathy in a glomerulus-on-a-chip microdevice. Lab Chip 17. https://doi.org/10.1039/c7lc00134g
41. Musah S, Mammoto A, Ferrante TC et al (2017) Mature induced-pluripotent-stem-cell-derived human podocytes reconstitute kidney glomerular-capillary-wall function on a chip. Nat Biomed Eng 1. https://doi.org/10.1038/s41551-017-0069
42. Roye Y, Bhattacharya R, Mou X et al (2021) A personalized glomerulus chip engineered from stem cell-derived epithelium and vascular endothelium. Micromachines 12. https://doi.org/10.3390/mi12080967
43. Perin L, Da Sacco S (2022) Generation of a glomerular filtration barrier on a glomerulus-on-a-Chip platform. Methods Mol Biol. 2373:121–131
44. Jang KJ, Mehr AP, Hamilton GA et al (2013) Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment. Integr Biol (United Kingdom) 5. https://doi.org/10.1039/c3ib40049b
45. Sciancalepore AG, Sallustio F, Girardo S et al (2014) A bioartificial renal tubule device embedding human renal stem/progenitor cells. PLoS One 9. https://doi.org/10.1371/journal.pone.0087496

46. Ng CP, Zhuang Y, Lin AWH, Teo JCM (2013) A fibrin-based tissue-engineered renal proximal tubule for bioartificial kidney devices: development, characterization and *in vitro* transport study. Int J Tissue Eng 2013. https://doi.org/10.1155/2013/319476
47. Jansen J, Fedecostante M, Wilmer MJ et al (2016) Bioengineered kidney tubules efficiently excrete uremic toxins. Sci Rep 6. https://doi.org/10.1038/srep26715
48. Weber EJ, Chapron A, Chapron BD et al (2016) Development of a microphysiological model of human kidney proximal tubule function. Kidney Int 90. https://doi.org/10.1016/j.kint.2016.06.011
49. Sochol RD, Gupta NR, Bonventre JV (2016) A role for 3D printing in kidney-on-a-Chip platforms. Curr Transplant Reports 3. https://doi.org/10.1007/s40472-016-0085-x
50. Homan KA, Kolesky DB, Skylar-Scott MA et al (2016) Bioprinting of 3D convoluted renal proximal tubules on Perfusable chips. Sci Rep 6. https://doi.org/10.1038/srep34845
51. King SM, Higgins JW, Nino CR et al (2017) 3D proximal tubule tissues recapitulate key aspects of renal physiology to enable nephrotoxicity testing. Front Physiol 8. https://doi.org/10.3389/fphys.2017.00123
52. Vormann MK, Vriend J, Lanz HL et al (2021) Implementation of a human renal proximal tubule on a Chip for nephrotoxicity and drug interaction studies. J Pharm Sci 110. https://doi.org/10.1016/j.xphs.2021.01.028
53. Baudoin R, Griscom L, Monge M et al (2007) Development of a renal microchip for *in vitro* distal tubule models. Biotechnol Prog 23. https://doi.org/10.1021/bp0603513
54. Jang KJ, Suh KY (2010) A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells. Lab Chip 10. https://doi.org/10.1039/b907515a
55. Cho S, Yoon JY (2017) Organ-on-a-chip for assessing environmental toxicants. Curr Opin Biotechnol 45:34–42
56. Vernetti L, Gough A, Baetz N et al (2017) Functional coupling of human microphysiology systems: intestine, liver, kidney proximal tubule, blood-brain barrier and skeletal muscle. Sci Rep 7. https://doi.org/10.1038/srep42296
57. Li Z, Su W, Zhu Y et al (2017) Drug absorption related nephrotoxicity assessment on an intestine-kidney chip. Biomicrofluidics 11. https://doi.org/10.1063/1.4984768
58. Nieskens TTG, Magnusson O, Andersson P et al (2021) Nephrotoxic antisense oligonucleotide SPC5001 induces kidney injury biomarkers in a proximal tubule-on-a-chip. Arch Toxicol 95. https://doi.org/10.1007/s00204-021-03062-8
59. Cohen A, Ioannidis K, Ehrlich A et al (2021) Mechanism and reversal of drug-induced nephrotoxicity on a chip. Sci Transl Med 13. https://doi.org/10.1126/scitranslmed.abd6299
60. Hwang SH, Lee S, Park JY et al (2021) Potential of drug efficacy evaluation in lung and kidney cancer models using organ-on-a-chip technology. Micromachines 12. https://doi.org/10.3390/mi12020215
61. Hansell P, Welch WJ, Blantz RC, Palm F (2013) Determinants of kidney oxygen consumption and their relationship to tissue oxygen tension in diabetes and hypertension. Clin Exp Pharmacol Physiol 40:123–137
62. Yasotharan S, Pinto S, Sled JG et al (2015) Artery-on-a-chip platform for automated, multi-modal assessment of cerebral blood vessel structure and function. Lab Chip 15. https://doi.org/10.1039/c5lc00021a
63. Tiong HY, Huang P, Xiong S et al (2014) Drug-induced nephrotoxicity: clinical impact and preclinical *in vitro* models. Mol Pharm 11:1933–1948

64. Tasnim F, Zink D (2012) Cross talk between primary human renal tubular cells and endothelial cells in cocultures. Am J Physiol Renal Physiol 302. https://doi.org/10.1152/ajprenal.00621.2011
65. Shin SR, Kilic T, Zhang YS et al (2017) Label-free and regenerative electrochemical microfluidic biosensors for continual monitoring of cell Secretomes. Adv Sci 4. https://doi.org/10.1002/advs.201600522
66. Stone HA, Stroock AD, Ajdari A (2004) Engineering flows in small devices: microfluidics toward a lab-on-a-chip. Annu Rev Fluid Mech 36. https://doi.org/10.1146/annurev.fluid.36.050802.122124
67. Sydney Gladman A, Matsumoto EA, Nuzzo RG et al (2016) Biomimetic 4D printing. Nat Mater 15. https://doi.org/10.1038/nmat4544

Lung-on-a-Chip

20

Brinda Poojary

Abstract

The human lungs are integral for gas exchange, regulation of blood pressure, protection, and maintaining homeostasis in the human body. The study into various diseases that affect the human lungs, and additionally drug development and toxicity studies, is currently conducted on nonhuman animal models (hereafter referred to as "animal models"). However, there is mounting evidence on the failure of animal models to translate human biology and provide human biology-based solutions to diseases and other toxicity issues. This has called for the development of relevant human biology-based models. The human lung-on-a-chip is one such model that mimics the physiology of human lungs and displays infinite potential to accurately imitate the human lung and aid in personalized medical research. This chapter details various aspects of the human lung and requirements for a model to be an ideal human lung model and dovetails the history and mechanism of the lung-on-a-chip with various case studies on the use of these models, culminating into a way forward for this technology.

Keywords

Lung · Lung-on-a-chip · Organ-on-a-chip · Animal models · Alternative to animal models

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20.1 Features of Human Lungs

The human lungs are a part of the lower respiratory tract which play a crucial role in gas exchange, protection, maintaining homeostasis, and regulation of blood pressure. Gas exchange is the major function of the lungs between the lungs and the blood [1]. This takes place through the blood-air barrier, which is a thin membrane (~0.5–2 μm thick) folded into around 300 million alveoli, resulting in a large surface area (~ between 70 and 145 m^2) conducive for gas exchange to occur [1, 2]. The cilia on the respiratory epithelium, which lines the respiratory tract, beats rhythmically and carries mucus, creating a mucociliary clearance which aids the lungs in removing particles like pathogens which may be inhaled and preventing them from reaching the delicate lung tissues [3]. Immunoglobulin A is secreted by the lining of the lung, protecting against respiratory infections [4]. Mucus secreted by the goblet cells contains antioxidants, defensins, and antiproteases which are antimicrobial compounds. The lining of the lungs also contains various immune cells like macrophages and dendritic cells (Fig. 20.1).

The respiratory epithelium lines the trachea, bronchi, and bronchioles. The epithelial cells of the human respiratory tract are broadly divided into three categories which are the ciliated columnar cells, basal cells, and the non-ciliated secretory columnar cells. The epithelium is ciliated and interspersed with ciliated cells, non-ciliated goblet cells, basal cells, macrophages, and terminal bronchioles-club cells. The working of the mucociliary clearance is regulated by the airway surface liquid which is secreted by the epithelial cells and submucosal glands that

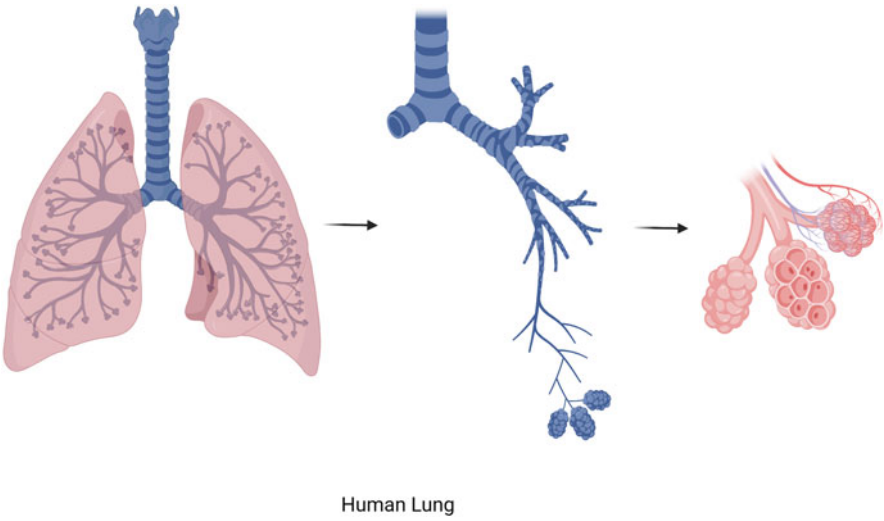

Fig. 20.1 Anatomy of the human lung, including the trachea, bronchi, bronchioles, alveoli, and the alveolar sacs

line the respiratory tract [5]. The bronchioles are further divided into alveoli made up of the alveolar cell and alveolar macrophage. Alveolar cells are classified into Type I and Type II cells which are also known as pneumocytes and make up the walls of the alveolar septa that separates adjacent alveoli [6]. Type I cells are made up of squamous epithelium cells lining the alveolar wall which enable easy gas exchange owing to their extremely thin walls. The type II cells, on the other hand, produce and secrete the epithelial lining fluid and the lung surfactant. Type II cells can differentiate into Type I cells which lack the ability to differentiate on their own [7].

In addition to these features, the human lungs also host many microorganisms which are known as lung microbiota which maintain homeostasis by interacting with the airway epithelial cells [8].

20.2 The Ideal Human Lung Model

An ideal human lung model needs to include the structural and supporting cells, where the changes in the various functions and activities of the non-epithelial as well as epithelial cells can be monitored and will also need to be able to model the airway mucins. The following few paragraphs will be able to provide the necessary justification for this statement (Fig. 20.2).

In the lungs, the epithelial cells face the lumen and are supported by the subepithelial and parenchymal tissues at the base [10]. The bronchial epithelium is made up of three cell types which are organized as a pseudostratified cell layer. The apical layer of the pseudostratified bronchi epithelium consists of secretory cells like the serous cells and goblet cells which play a key role in the secretion of mucus in the airway. Among these, the goblet cells are found in abundance in the conducting airways and are the main producers of airway mucus. The goblet cells show microvilli expression on their cell surface and possess electron-lucent secretory granules. They are an integral part of the inflammatory response wherein they quickly increase mucus secretion on exposure to pathogens and other irritants [11, 12]. A healthy human lung contains a thin lining of mucus that lines the

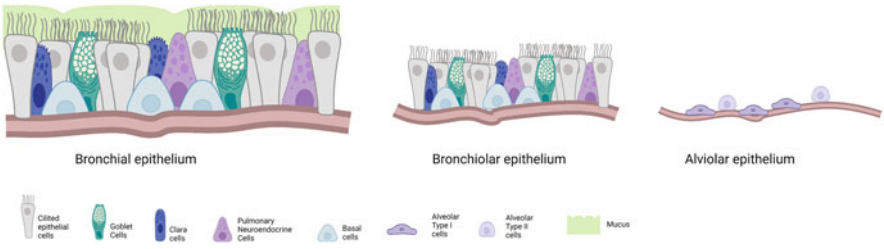

Fig. 20.2 Changes in the epithelium from bronchi to bronchioles to alveoli. (Adapted from Marshall et al. [9])

epithelium which is essential for the innate defense mechanism. The mucus layer aids in trapping any foreign particle that enters the lungs that can be potentially harmful for the body and prevents it from entering the alveoli. Serous cells are similar secretory cells which are observed to be present in the small airways of the human lungs [13] and have a granule content that is electron dense.

Fifty percent of all the epithelial cells in the airways are made up of ciliated cells. These cells reach the lumen and are attached to the basal membrane. They are the key feature of the mucociliary clearance wherein the beating movement of their cilia drives the mucus towards the pharynx along with the particles that the mucus may have trapped, and thus getting them out of the airways [14]. The ciliated epithelial cells and the columnar epithelial cells form tight junctions in the pseudostriated layer forming desmosomes which are specialized adhesive protein complexes that aid in their attachment to the adjacent and basal cells [10, 15]. The lumen of the airways is separated from the underlying tissue with the help of this selectively tight barrier that is formed in the paracellular space between the epithelial cells. A belt-like structure is formed by these tight junctions formed by closely connected cells and is closest to the lumen [15]. These play an important role in the selective permeability of the barrier which protects the airways from inhaled particles that may be pathogenic or toxic [16].

Basal cells are speculated to have a progenitor role in the airway epithelium [17]. Furthermore, they are known to secrete cytokines, chemokines, and growth factors. The basal cells which aid in the attachment to superficial cells to the basal membrane, in fact, do not reach the lumen. These cells not only play a role in the attachment of the columnar epithelial cells to the basement membrane, but also in injury repair and defence [18]. The composition of the basement membrane, also known as the lamina propria, is made up of proteoglycans, collagen, and elastins. This structure forms a kind of a scaffold on which the basal epithelial cells are attached [19]. This membrane enables the interaction between the subepithelial pulmonary fibroblasts and the epithelium [20]. The pulmonary fibroblasts are the most abundant cells in the lung, making up 40% of all the lung cells [21]. These fibroblasts which were once speculated to only aid in support and ensure the deposition of the components of the basal membrane within the lungs [22] are now understood to contribute to inflammation actively and directly [23].

The pulmonary fibroblasts, epithelium, extracellular matrix, and the neural tissue make up the mesenchymal trophic unit which is shown to play an important role in the growth and branching of airways during lung development [24, 25]. Any form of dysregulation of the cellular components here can lead to pathological consequences. Immune cells like alveolar macrophages, neutrophils, eosinophils, mast cells, and dendritic cells are also found in the lungs, which can reach the airways through the basal membrane to not only aid epithelial cell function, but also provide protection against inhaled substances [14].

20.3 Diseases of the Human Lungs

The human lungs are prone to various disorders and diseases. Human lung diseases can be grouped under three broad categories which are airway diseases, lung tissue diseases, and lung circulation diseases. The airway diseases include the ones that affect the airway passages that carry gases into and out of the lungs. These forms of diseases lead to narrowing of the airways and are diseases like asthma, bronchiectasis, COPD, etc. Airway obstruction is caused by inflammation in the bronchial tree. The constriction, because of this inflammation, prevents the entry of sufficient air into the alveoli. A variety of diseases like asthma, chronic bronchitis, bronchiectasis, and Chronic Obstructive Pulmonary Disease (COPD) are caused by obstructions in the airway and are called obstructive lung diseases. The causes of asthma are yet to be understood completely [4]. Bronchiectasis is caused by severe infections and due to cystic fibrosis. Chronic bronchitis is commonly caused due to smoking. The breakdown of the alveolar tissue causes emphysema, and the increase in the severity of this could lead to the development of COPD. The constant stress from smoking can disturb the airway basal cells which then lose their regenerative ability and hinder the repair of the epithelial barrier. Continued stress can lead to a malignant transformation of the basal cells, deranging them, transitioning into COPD [26].

Human lung tissue diseases hamper the lung tissue structures leading to scarring of the tissue or tissue inflammation which restricts the lungs from full expansion, hindering oxygen intake and carbon dioxide release. On being attacked by pathogens, the immune system responds through inflammation of the region. Inflammation in the respiratory system leads to pneumonia when lung tissues are inflamed, bronchitis and bronchiolitis when the respiratory tract is inflamed, and pleurisy when the pleurae around the lungs are inflamed. Alcohol too causes inflammation of the lungs and causes inflammatory lung disease. Long-term exposure to alcohol affects the mucociliary clearance by desensitizing ciliary response and thus reducing the mucociliary clearance. When the mucociliary clearance is reduced, there is a significant decrease in macrophages. This leads to an inflammatory response that triggers the release of cytokines and increases the susceptibility to infections [27, 28].

Diseases like pulmonary fibrosis are caused when the lung tissues are inflamed for a long period of time. They are characterized as restrictive lung disease due to the restriction of the amount of lung tissue that plays a role in respiration. A fibrous connective tissue replaces the functional lung tissue which leads to fibrosis and is caused by various lung diseases like autoimmune diseases or a variety of occupational lung diseases. The human lung circulation diseases affect the blood vessels in the lungs. When there is any form of inflammation of the blood vessels, or clotting, or scarring, it leads to lung circulation diseases. This too affects the lung's ability to uptake oxygen and release carbon dioxide. Pulmonary hypertension is an example of this kind of disease where even the heart function is affected. Pulmonary embolism is a type of lung circulation disease where a blood clot gets lodged in the pulmonary arteries. This affects the blood supply of the lungs. Chest traumas cause lung contusion which results in the alveoli hemorrhaging, causing fluid build-up which harms breathing. Compression of the lungs from fluid in the pleural cavity called

pleural effusion, or by air called pneumothorax, or blood called hemothorax can affect lung function.

Another major disease of the human lungs is lung cancer. Lung cancer could directly arise from lung tissue or could be caused due to cancerous cells metastasizing from any other part of the body. Small-cell and non-small-cell lung carcinomas are two main types of primary tumours of the lungs. The small-cell carcinoma is majorly seen in the lungs as compared to other organs and is a malignant cancer. It is characterized by its high growth fraction, short doubling time, and early development of metastases. In comparison to this, the non-small-cell lung carcinoma is epithelial lung cancer and accounts for 85% of all the lung cancers. Pulmonary hypoplasia, cystic fibrosis, infant respiratory distress syndrome, and congenital diaphragmatic hernia are all forms of congenital lung disorders.

20.4 Limitations of Current Use of Animal Models for Human Lungs

Non-human animals (hereafter 'animals') are the most used models for studying the basic biology of humans, human diseases, drug research, development of drugs, and toxicity and safety testing. However, various conditions like the effects of laboratory environment, species differences with respect to genetics and physiology, innumerable disparities between the human disease and the animal models used to model these diseases play a role in undermining the reliability of animals to provide information on human health.

The external factors in the laboratory, like windowless rooms, artificial lighting-restricted housing environments, human noise, etc., play a key role on the animals' physiology and behaviours. These can prevent the animals from expressing their species' typical behaviours, which ultimately cause them distress [29, 30] and contagious anxiety [31]. Human interaction for tests too is key in interfering with results. Monkeys have shown to produce increased cortisone levels when they are restrained to collect their blood [32]. Studies have also demonstrated that rats experience an elevated heart rate and blood pressure when they watch other rats being decapitated [17]. There are also prolonged and significant elevations in stress levels observed in animals when they are caught and removed from their cages during routine animal experimentation, in addition to what is caused during experimental procedures [17]. This increase can also interfere in test results [33]. Different conditions also alter the genetic expression, neurochemistry, and nerve regeneration in animals [34].

In addition to these external factors affecting animal tests, internal physiological factors too significantly alter results. Although some animals may be genetically close to humans, gene regulation substantially differs from species to species. This means that even if there is a high degree of genome conservation, the function and order of the genes among species can critically differ. This can result to different outcomes when the order in which the genes are expressed differs. This lack in congruence between animal models and humans also deters the translational

reliability of these models. Most human diseases do not naturally occur in animals and need to be artificially induced in the animals, which is extremely difficult considering the complexity of human diseases and thus does not prove very useful [35, 36]. There is a growing body of evidence of the high rate of clinical failure in drug development partially due to the lack of predictability of animal models [37]. The FDA, in 2004, estimated that 92% of the drugs that clear preclinical trials, which include animal tests, fail to enter the market [38]. Since then, no matter the efforts put in to improve the predictability of animal testing, the failure rate has now increased to 96% [39], where the lack of safety and effectiveness was not predicted by animal tests [40].

20.5 Animal Models for Human Lungs

Mice have come to become the most used animals to understand human lungs because of their short reproductive cycle, the availability of reagents, and the deep understanding into their immunologic system, among other reasons [41–43]. However, they do not exhibit the appropriate phenotype to mimic the human lungs and is considerably different in structure. The lung capacity of a mouse is 1 ml compared to 6000 ml of a human. While the human right lung has 3 lobes and left has 2, the mouse right lung has 4 and the left lung has 1 lobe. The pleura in mice is thin with the ability to withstand high pressure. The parenchyma occupies slightly more space in the mouse lung than in human lungs, while the alveoli in mice are smaller than that of humans and the blood gas barrier too is smaller in the lungs of mice compared to humans. This plays a critical role in gas exchange as well as parenchymal lung mechanics. The respiratory bronchioles in mice are fewer than in humans. Mice display monopodial branching of the bronchus as opposed to the human dichotomous branching of the bronchus which is a significant difference.

In addition to the general anatomical differences, animals also exhibit major variations in the architectural organization of the lungs, cellular composition of the respiratory tract, distribution of the connective tissue, to name a few. The submucosal glands are found only in the proximal trachea of the mice, but they are found much more abundantly in the human lung, penetrating deep into the lungs. There is significant difference that is also observed in the cellular composition of the airways. As mentioned before, the pseudostratified epithelium is composed of ciliated, basal, secretory, neuroendocrine, and intermediate cells. The secretory goblet cells in human lungs are way more abundant than adult mice maintained in controlled laboratory conditions. The respiratory epithelium of mice is also significantly thin compared to humans and they also have a relatively large lumen as opposed to humans [44, 45]. Clara cells are present in large numbers and there is an absence of submucosal glands in mice lungs compared to human lungs [45]. Using mice as models for human lungs could impede the understanding of inflammatory processes that compromise lung function in humans considering that mice have relatively large airway size, in addition to the lack of mucous glands in the lungs of mice [46].

These noteworthy differences between animal models and human lungs have also impacted disease studies like asthma and COPD. Guinea pigs are the most used animal models for studying allergic respiratory diseases. However, among other concerns with using these animals, the most notable would be the axon reflexes that are seen in these animals which have not yet been described in human airways [47]. Mice are used largely to study asthma even though they do not develop asthma spontaneously, which must be artificially induced in them. They are used to study the targets that are responsible for controlling allergic inflammations [48]. Acute and chronic models are developed for this purpose. It has been observed that the pattern and the distribution of the pulmonary inflammation are different than what is seen in human patients [49]. While the inflammation in human asthma persists and the symptoms can recur on new exposure to the allergen, the acute mouse models show that the airway inflammation tends to resolve in a few weeks after antigen exposure [50]. Asthma, like many other diseases which are specific to humans, does not have any efficient animal that can be used as a model to depict the features and phenotypes that are typical to the human disease [51].

The inherent differences between animals and humans pose as drawbacks even in the field of drug discovery and research. The translatability from animals to humans is extremely poor despite the extensive development of various pharmacological interventions, be it anti-tumour necrosis factor therapy, steroid treatment, or surfactant replacement. An example of this is in the case of mice used as models of Acute Lung Injury (ALI). When comparative proteomic analyses were performed between the bronchoalveolar lavage fluid from an ALI patient with that of mice, there were 21 homologous proteins that were identified [52]. Significant differences have also been noted in the pleural anatomy [53], while the normal rates of alveolar epithelial fluid transport have also been observed to differ across species [54–56]. There is a lack of robust biomarkers in animal used as models of ALI that could translate into the human syndrome which poses a critical issue for drug discovery research [57].

20.6 History of Lung-on-a-Chip

As mentioned early in this chapter, the major function of the human lungs is that of gas exchange. The Air-Liquid Interface (ALI) exists in the sponge-like structure of the human lungs between the inhaled air and the vascular system across the pseudostratified membrane. The alveoli subunits in the lungs maximize the surface area for gas exchange. This ALI is where the inhaled air along with the foreign particles and pathogens encounters the inner body. Thus, the ALI is the key feature of the lungs that is used to understand human lung behaviour and pathology. There has been immense progress over the years in the field of 3D cell culturing methods with the use of microfluidic techniques and improved apparatus, so much so that these methods have been enhanced in a way that they are able to approach the recapitulation of organ-level functions in vitro. There is a possibility of introducing continuous perfusion—a process where the exchange of oxygen and other molecules is accomplished across semipermeable microvascular walls by forcing blood

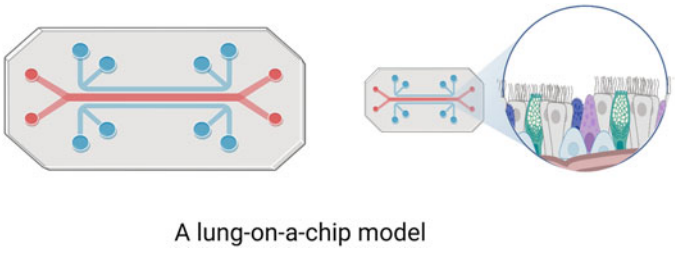

Fig. 20.3 A lung-on-a-chip device

through microscopic vessels that are within the biological tissue. The cultured cells are subjected to imposition of controlled fluid and solid mechanical stresses in addition to the perfusion of media. This aids in mimicking the biomechanical and biochemical microenvironments that are found in vivo. The lung exhibits an immensely mechanical environment during breathing where the tissue stretches owing to the forces imposed on the cells. This is mimicked through the capabilities of the microfluidic system (Fig. 20.3).

The microfluidic device that paved the way for lung-on-a-chip platforms was first pioneered by Huh et al. in 2010 [58]. Three layers composed of Polydimethylsiloxane (PDMS) were organized in a way that two layers with parallel micropatterned channels had a thin and porous membrane running between them. A two-chambered membrane separated system was created due to this porous membrane. The membrane was then coated with ECM on both sides to enable cell attachment. One side of the membrane had alveolar epithelial cells seeded to the ECM, while the other side was seeded with vascular endothelial cells. To establish an ALI, the epithelial side of the chamber had a system for flowing air over it, which mimicked the cell make-up and the structure that is observed in the human alveolus. The mechanical stimulation required to mimic breathing was established by using two lateral channels that ran parallel to the epithelial and endothelial channels, through which vacuum was applied leading to the deformation of the flexible PDMS. This led to a controlled cyclic stretching of artificial alveolar membrane which was at par with the in vivo amplitudes and frequencies. This stretching of the membrane was conducted when epithelial layer had to be biomimetically aligned and during cell culture. In order to confirm if the inclusion of mechanical strain on the alveolar cells changed the lung cell behaviour in lung-on-a-chip models, an immunological standpoint was considered for investigation. When an inflammatory cytokine was introduced into the endothelial side of the membrane (which is the vascular channel) and the endothelial expression of the intercellular adhesion molecule-1 (ICAM-1) was checked, it was observed that there was no measurable difference in the immune response with and without the mechanical actuation of the membrane. On introducing silicon nanoparticles (commonly used in airborne nanotoxicity research [59]) on the epithelial side of the membrane (which is the alveolar channel of the device), however, it was noticed that there was a significant

difference in immunological response which depended on mechanical actuation of the membrane. There was an increased immune response that was observed in the presence of this mechanical actuation which was also accompanied by an increase in the trans-membrane migration of the nanoparticles as opposed to the observations in the device which was not subjected to the mechanical actuation.

This system ensured the formation of junctions between adjacent cell types, the production of pulmonary surfactant, and a physiologically similar barrier porosity.

20.7 Important Features for Lung-on-a-Chip

An ideal ALI model should have the ability to serve as a cell culture substrate which promotes cell attachment, differentiation, proliferation, and interactions, and it should also be able to replicate the features of lung tissue like gas exchange, ciliary beating, and surfactant production. Cell behaviour, like adhesion and migration, are affected by a multitude of physical properties like topography, porosity, membrane dimensionality, swelling; these physical properties also affect the barrier function like permeability and leaking. Thus, it is important to maintain a high control over these various physical features in order to fine-tune the ALIs and perfect specific functions.

The porosity of the membrane is crucial for the permeability of gas and nutrients. It plays a key role in modulating the migration ability and the interaction between the different types of cells which are seeded on each side of the membrane [60]. There are commonly used porous networks like polyethylene terephthalate (PET) or polycarbonates which are available in different pore sizes and can be used to mimic the ALI and are introduced into the PDMS through techniques like freeze-drying, particle leaching, or gas foaming. The physiological microenvironment of cells is seldom smooth and flat. The spatial cues which are expressed by the surface topology are what modulates attachment of cells along with their alignment and spreading. The topology of the ALI in a lung-on-a-chip thus needs to be tuned in a way to match the different convoluted textures of the tissues like that of the respiratory membrane which exhibits a large surface area and a high curvature. This can be achieved through lithography or etching to make the material surfaces smooth or patterned or nano-roughened or customized as per requirements [61]. The spacing and height of the micro-grooves, the parallel/semi-aligned/random orientation, and the rectangular/rounded edges are the parameters that are capable of being tuned.

To make sure that the multilayer geometry of the native cell microenvironments is modelled well, the dimensionality of the scaffolds can be increased. It has been observed that the formation of cell-cell interfaces and tissue organization can be enhanced using 3D scaffolds. Techniques like electrospinning can be used for creating fibrous and porous networks that mimic the in vivo ECM scaffolds, while flow rate can be modulated to control swelling, fibre thickness, and material porosity [62]. The type of polymer and cross-linker that is used, along with the density of cross-linking, determines the degree of swelling or the contraction. In addition to the

physical properties that affect the ALI of lung-on-a-chip, the biochemical properties of the design need to be factored in too, considering the importance that chemical and biological stimuli like cell adhesion molecules, other cells, drugs, growth factors, etc. have been observed to play a significant role in the regulation of cell behaviour [63]. Incorporating bioactive compounds and proteins, tuning the substrate's hydrophobicity, or the incorporation of functional groups to improve the growth and functionality of the cells cultured at the ALIs can be achieved by modifying the ALIs. It must also be borne in mind to carefully choose the substrates so that their degradation does not lead to by-products that are cytotoxic or which may adversely affect various properties of the substrate like its porosity, topology, or its mechanical properties.

It is crucial to tune the surface hydrophobicity because fluorescent drugs or dyes and similar small molecules may non-specifically adsorb on the surface. This, however, can be avoided by coating the hydrophobic polymers like PDMS with non-ionic surfactants, poly-Lysine, immobilizing or adsorbing ligands, functional groups, or proteins. Treatment with ECM proteins like collagen and fibronectin enhances the cell biocompatibility of the PDMS and other substrate materials made of synthetic polymers [58]. These ECM proteins enhance and promote the proliferation and attachment of cells. The PDMS surface can be adsorbed with proteins from a protein solution through micro-contact printing or coating. The proteins can also be immobilized on the PDMS by treatment with cross-linkers like PEG-based linkers or glutaraldehyde, or by plasma activation, among other methods. Functional groups like phosphate, carboxyl, methacrylate, thiol, amoxyl, and butyl groups aid in adsorption or/and sequestration of biomolecules like growth factors and glycoproteins and enhance cell growth when they are introduced either by polymerization methods like chemical modification or surface plasma activation, or by grafting [63].

The mechanical stresses applied to the lung-on-a-chip aid in cell growth and tissue functions and so must be taken into consideration for the design in addition to the physical and biochemical factors [64]. As mentioned previously, the uptake of nanoparticles across the air-blood barrier in the alveolus is influenced by the mechanical stress applied during breathing [65]. Substrate stress-relaxation regulates the proliferation of cells and their spreading [66] and affects scaffold remodelling [67], while substrate stiffness has been shown to affect the lineage of stem cell differentiation [68]. Altered matrix stiffness is observed in several diseased states like fibrotic tissue, cancer, and emphysema which affect the cell fate in the progression of the disease, repair, and remodelling [69]. The mechanical tunability of the lung-on-a-chip can be applied to customize and enhance the functionality of the model based on the research or study. The PDMS is made up of flexible monomer chains which are cross-linked at intervals and cured till they become relatively soft [70], exhibiting low energy dissipation [71]. PDMS is the most suitable polymer for lung-on-a-chip as it proves to be favourable for the application of mechanical stimulation due to its tunable stiffness compared to other types of permeable membranes like PLA, PC, and PET [72].

Mechanical forces of the tissues generate most responses from the fibrous ECM proteins, like the elastic extension and recoil, tensile strength, etc. It is observed that different tissues have structural proteins of different compositions in vivo, and this reflects what they require physiologically. This is achieved in the lung-on-a-chip models by blending the PDMS with ECM proteins which can modulate its mechanical properties so that it can be used as an ALI. To summarize this information, lung-on-a-chip models use a microfluidic channel to simulate the trachea and blood vessels. The flow rate of the fluid sheer stress of the bloodstream and the flow rate of the air are controlled [73]. The microchannel of the simulated trachea and the simulated blood vessels are separated by placing membrane materials between them which also act as the base to support cell culture and simulate respiratory movement by realizing the stretch stress which are applied during model construction using fluid mechanics, stretch stress, etc. [74]. The real physiological structure of the human lung is thus mimicked by the lung-on-a-chip model; the alterations of the required settings of the parameters like the fluid flow rate, stress stretch frequency, size, etc. [48, 75]

20.8 Current Applications

The lung-on-a-chip can be used to simulate and study human lung diseases in addition to simulating the microenvironment of the human lungs.

20.8.1 Lung Injury

In the case of lung injury, the lung-on-a-chip model includes a mechanical stretch model, a shear force model, and one that includes both [76]. For the shear force model, there have been studies conducted to understand the mechanical damage to small airway epithelial cells which is simulated using a limited length liquid plug. This plug is formed in the microchannel of the microfluidic chip. A blocking fluid of 1 pl is transported by the generator of the microfluidic chip to the microchannel where the epithelial cells are cultured on the gas-liquid interface. The liquid plug is ruptured when the plug is diffused and the liquid flows out. This is measured using an auscultator. It has been observed that when the liquid plug is regularly ruptured, it results in cell death. A study conducted by Tavana et al. [77] used this model to understand the damage of the pulmonary epithelial cells which is caused by the deposition of airway surface liquid during the arousal of occluded liquid plugs in the airways. On account that the pulmonary surfactant plays a significant role in abating the detrimental effect of reopening stresses and the fact that the airways in the peripheral lung are susceptible to closure at low lung volumes, any sort of dysfunction of the pulmonary surfactant in the case of different lung diseases increases the chances of these detrimental effects by destabilization of the pulmonary surfactant, giving rise to occluding liquid plugs in the airways. The study found significant cell damage during the flow of the liquid plug on the pulmonary epithelial cells, and that

the airway surfactant could effectively prevent cell death. Through this study, it was inferred that surfactant could potentially be regarded as a cure for the treatment of various lung diseases like acute respiratory distress syndrome. In yet another study, Douville et al. studied the mechanical stretch model by designing a lung-on-a-chip model to mimic the mechanical stretch that the human alveolar air-blood barrier experiences [76]. Pathological edema and physiological inflation have been simulated in lung-on-a-chip by changing the liquid load on the alveoli that is mimicked by the chip. Thus, many such environmental factors like solid and fluid mechanics have been explored to simulate various conditions of lung injury including those caused by a respirator [78, 79].

20.8.2 Pulmonary Inflammation

Bacterial, viral, fungal, and other pathogenic infections cause pulmonary inflammation in human lungs. Inflammation in the lungs can also be caused by radiation, allergic or chemical pneumonia. These lead to various responses like exudation and aggregation of white blood cells, the expansion of capillaries, etc. The lung-on-a-chip model has been used to simulate inflammatory responses like these. Studies have been conducted where pro-inflammatory cytokines and bacteria have been introduced into the alveolar microchambers in the air-blood barrier of the lung-on-a-chip model [58, 80]. The intercellular adhesion molecule-1 was observed to be expressed by the vascular endothelial cells present on the vascular side of the membrane. The formation of capillaries was promoted to simulate the adhesion of neutrophils in the channel on the vascular endothelial cells to recruit neutrophils. It was then observed that the neutrophils entered the alveolar chamber by penetrating the vascular endothelial cells and membrane, thus leading to phagocytosis of the bacteria through a directional migration of the neutrophils.

20.8.3 COPD and Asthma

There are two major phenotypes that characterize COPD—emphysema and small airway disease. Emphysema entails the enlargement of air spaces and the destruction of lung parenchyma. Small airway disease encompasses the accumulation of inflammatory cells, progressive subepithelial fibrosis, and the exudation of mucus in the airway lumen. The airway-on-a-chip model has been developed where a living bronchiole biomimetic system mimics the small airway disease that precedes the development of emphysema [81]. The development of this technology has facilitated recapitulating many of the relevant features of COPD like the aggravation caused by the viral and bacterial infections, the increase in neutrophil recruitment, the hypersecretion of selective cytokines, etc. on a lung-on-a-chip [81, 82]. This model was successfully tested for its efficacy to measure therapeutic responses from multiple donors of exacerbated COPD. The study identified that macrophage colony-stimulating factor (M-CSF) acted as a biomarker for exacerbations when they

stimulated the airway-on-a-chip model constituting either of non-diseased or individuals suffering from COPD with Poly[I:C] (viral-like stimuli) or LPS endotoxin (bacteria-like stimuli). M-CSF was noticed to upregulate upon viral-like stimulation in COPD mimicking airway-on-a-chip. A 'breathing-smoking lung-on-a-chip' was modeled where the effects of whole cigarette smoke were analyzed by delivering cigarette smoke over human mucociliated bronchiolar epithelium under conditions that were relevant to the human physiology which mimicked breathing. The study using this model led to the discovery of a set of disease-specific biomarkers—a few of them which were never associated with COPD; and two smoke-induced ciliary micro pathologies [83].

These systems have also been used to model asthma conditions by adding asthma-inducing immune factors. These advances have not only made it easier to study inflammatory diseases over a span of several weeks to enhance the insights into disease mechanisms, but also have helped screen new therapeutics [84]. The team at the Wyss Institute provided the proof-of-principle for the use of the small airway-on-a-chip as a platform for discovery of disease-specific drugs and biomarkers. The team, in collaboration with industrial partners like Pfizer and Merck Research Laboratories, demonstrated two drugs which targeted different key molecular components of the inflammatory pathways had the potential to suppress the pathological processes in small airway chips which were tailored for asthma and COPD studies [83]. These models were used for the validation of reports on a drug used for rheumatoid arthritis, tofacitinib, which was shown to suppress lung inflammation compared to the inefficiency of dexamethasone [81].

20.8.4 Cystic Fibrosis

The current preclinical models of Cystic Fibrosis (CF) lack reliability due to their inability to mimic the structural, immunological, and bioelectrical features that are found in the human lungs exhibiting CF. This has impeded the development of new therapies for the disease. Lung-on-a-chip model has been used to study CF. Plebani et al. cultured primary bronchial epithelial cells (HBE) from healthy people and people with CF to create health and CF airway chips [85]. When both the phenotypes were compared, it was observed that the epithelium and the endothelium of healthy and CF individuals formed tight monolayers that covered the entire length of the channels after 12–14 days of culture under the ALI. The CF airway chips displayed higher number of ciliated cells as opposed to the healthy chips after 2 weeks in the ALI in addition to a higher Ciliary Beat Frequency (CBF) compared to the healthy chips, consistent with the data observed in vivo [86]. Like the enhanced inflammation found in vivo in CF lungs, the CF chips displayed a considerable increase in pro-inflammatory cytokine IL-8, no difference in IL-6, and decreased levels of IP-10, GM-CSF and MIP1 compared to the chips with healthy cells after 2 weeks in the ALI. To mimic the frequent infection of P. aeruginosa in the CF airways [87], the chips were inoculated with GFP-labelled P. aeruginosa bacteria. Large clusters of GFP-labelled bacteria were detected in the mucus layer which was closely apposed

to the underlying epithelium in the CF chips, and a high number of bacteria were observed in the CF airway chips as opposed to the healthy chips through fluorescence densitometric quantitation. This observation is consistent with the observations in vivo [88]. There was also a significant increase in IL-6, TNF-, and GM-CSF levels and no increase in IL-8 levels that was observed in the vascular effluents of the CF airway chips and the healthy chips because of bacterial infection. This model also opens the possibility for more personalized preclinical models for mechanistic and drug testing and discovery studies. This can advance the understanding of CF and help increase the delivery of efficient and effective therapeutics to patients.

20.8.5 Pulmonary Fibrosis

Lung-on-a-chip models have been used to model the progression of fibrosis in the human lungs. Using a technique called microlithography, which is used to print electronic chips, micro silicon-based pillars were created by a research group [89]. These micropillars were then coated with lung fibroblasts or mixtures of human lung small epithelial cells, which spread over the micropillars and arranged themselves in structures that were like the human lung alveoli. TGF-beta, which is a pro-fibrotic factor, was added to the system and was observed to induce fibrosis within a week. This model was then used to study the efficacy of anti-fibrosis treatments like Esbriet and Ofev. It was observed that although the therapies inhibit fibrosis at different degrees, they both seemed effective in preventing stiffening of the tissue. These observations through such microfluidic lung-on-chip models have helped understand the potential biomechanical mechanism of action of Ofev and Esbriet on fibrosis treatment. Another study [90] was conducted to develop a lung-on-a-chip model that could reproduce the alveoli and study the development of pulmonary fibrosis where the scar tissue that covers the alveoli prevents gas exchange. The chip is comprised of a gold wire mesh which serves as a scaffold for cell culture. The technology has the potential not just for basic research, but also to screen compounds for drug development and identify the best treatment based on personalized precision medicine by modelling human lung diseases effectively.

20.8.6 Cancer

Multiple kinds of lung cancer, like small cell carcinoma and non-small cell carcinoma (adeno carcinoma, squamous carcinoma, large cell carcinoma), have necessitated the use of diverse medication, chemotherapeutic drugs, and increased drug resistance. The need for personalized diagnosis and treatment is the need of the hour to ensure symptomatic medication. Lung-on-a-chip models could be used to address the issues of comprehensive investigation into metastasis monitoring, long term culture, assessment of drug sensitivity of tumour cells, etc. A study was conducted to develop a lung-on-a-chip built with poly (lactic-co-glycolic acid)

nanometer electrospinning used as the backing material. This was used to form 3D co-culture of lung fibroblasts, vascular endothelial cells, and lung tumour cells. An anti-tumour drug—gefitinib—targets the epidermal growth factor receptor and lung cell A549. It was found that the drug broke through the barrier of the blood vessels leading to endosmosis [91]. A drug development study for cancer used a lung-on-a-chip model to understand the IL-2-induced drug toxicity in cancer patients which led to pulmonary edema [80]. The study found that the mechanical forces of respiration combined with IL-2 augmented the opening of the intercellular junctions of the alveolar epithelium and the endothelium. It was observed that the conjunction of mechanical forces with IL-2 led to a threefold increase in the permeability of the tissue over a span of 8 h as opposed to that caused only by IL-2. The co-administration of angiopoetin-1 was observed to aid in blocking the increased vascular leak. TRPV4 channels which were activated due to the mechanical strain increased the exudation of fluids from the lung capillaries. This was managed by testing the effect of a TRPV4 inhibitor which prevented the fluid leak caused by IL-2 administration along with 10% cyclic mechanical strain. The lung-on-a-chip model has also been used to develop an accurate, efficient, and high-throughput drug-screening platform to screen anti-cancer drugs that are used for the treatment of malignancies of the lung. The scope of individual therapy was demonstrated by testing single and combined chemotherapeutic regimens. The model turned out to be time- and resource-effective using less amounts of sample and proved to be highly sensitive [92].

20.8.7 COVID-19

The most recently significant application of the lung-on-a-chip model was to identify existing drugs for SARS-CoV-2 virus which could be repurposed to treat patients. The Airway Chip developed by the Wyss institute which consists of human lung airway cells differentiated into specific cell types that are found in the human airway was used for this purpose by the research team. The airway chip cells have high levels of Angiotensin Converting Enzyme—2 (ACE-2) receptor protein, which not only plays a key role in the physiology of human lungs, but also was used by the virus to infect cells. The team designed a SARS-CoV-2 pseudovirus which expressed the spike protein to identify the existing drugs that could inhibit the spike protein's ability to the ACE-2 receptor of human lung cells. Drugs like amodiaquine, clomiphene, hydroxychloroquine, arbidol, amiodarone, verapamil, chloroquine, and toremifene, which have already displayed activity against related viruses, were perfused in the airway chip's blood vessel channel in clinically relevant doses to mimic the drug distribution in the human body. This was followed by the introduction of the pseudovirus into the air channel of the airway chip 24 h later, thus mimicking the infection caused by the airborne virus through coughing or sneezing. Out of the drugs tested, amodiaquine, clomiphene, and toremifene were found to significantly prevent viral entry without damaging the cells in the chips. Amodiaquine reduced the infection by 60% and was found to produce more distinct

and broad protein changes than the rest of the drugs, proving to be a lead drug candidate [93].

20.9 Way Forward and Future of Lung-on-a-Chip

The lung-on-a-chip model, while being a momentous milestone on the path of scientific advances, has a few challenges to overcome. The use of PDMS for the fabrication of these devices is common; however, this material tends to adsorb hydrophobic drugs posing an issue during the assessment of the effects of drug solutions which have a high surface-to-volume ratios [94]. The accuracy of replication of the basement membrane of the blood vessels (300–400 nm) also needs to be addressed while simulating vascular and epithelial interfaces where currently the membranes have a thickness of ~10 mm. This could be addressed using 3D bioprinting inks or hydrogels to form better tissue structures and interfaces [95]. The cell-to-liquid and the surface-to-volume ratio is another area that needs to be addressed. The secretions and metabolites are diluted because of the volume of the media being larger than the volume of the tissues which could impact the cells. The surface-to-volume ratio plays a role in autocrine and paracrine signalling [94]. It is of key importance to identify the physiological hallmarks of each individual organ and have a universal standard for the fabrication, design, and utility of the device based on them. The mathematical modeling and use of advanced biosensors need to be improved to accurately reproduce physiological PK-PD so that they can be accepted for preclinical use. Additionally, there is a huge potential to explore the development of different parts of the respiratory system like the nasal cavity, larynx, pharynx, trachea, pleura, surrounding lymph nodes, etc., considering that each organ has its distinguishing anatomy and physiology. This deep dive into the study of different organs of the respiratory tract will enhance the understanding of the pathophysiology of different diseases affecting it and improve drug development and discovery. With respiratory diseases on the rise, and the growing rates of treatment failures, relapses, and drug resistance, the lung-on-a-chip model can be used to accelerate the development of personalized medicine by developing cells obtained from individual patients or acquiring them from a genetic profile subpopulation. These personalized models can then be used to carry out specific drug testing and therapies to advance individualized treatments. 'Personalized lung-on-a-chip' models developed from patient-specific cells and tissue samples or cells from a specific genetic group can be utilized to understand the physiology, genetics, and biometric parameters of the specific patient or genetic population and to develop and evaluate effective treatment strategies [96, 97]. Merging the lung-on-a-chip model with human stem cell engineering could aid in the understanding of rare congenital respiratory diseases. The microenvironment and mechanical cues that play a role in the differentiation, patterning, and organogenesis of stem cells can be controlled in the lung-on-a-chip model, and the use of human pluripotent stem cells (hPSCs) could make the studies more cost-effective while avoiding the invasive procedures that are used to obtain primary lung tissue samples [98–101]. This could help with

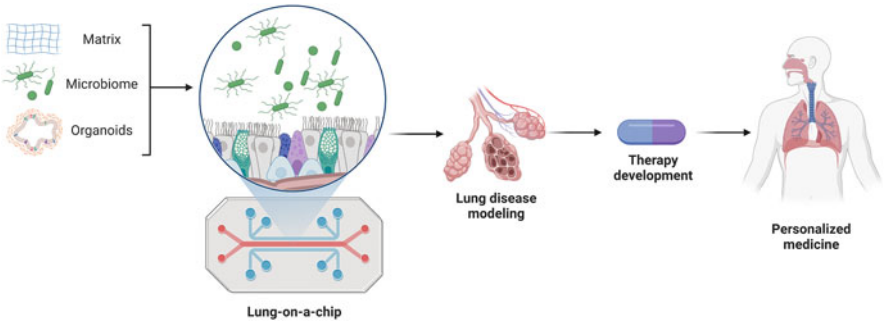

Fig. 20.4 Complex lung-on-a-chip for personalized medicine

new perspectives for patients who suffer from irreversible lung injuries and aid in the development of lung repair and regenerative medicine (Fig. 20.4).

The organ-on-a-chip market size is projected to reach USD 1020 million at a compound annual growth rate of 53% by 2027 with the top contenders in the field being companies like Organovo Holdings Inc. (US) Emulate, Inc. (US), TissUse GmbH (Germany), HemoShear Therapeutics, LLC (US), Nortis, Inc. (US), CN Bio Innovations Limited (UK), TARA Biosystems, Inc. (US), Axosim (US), MIMETAS BV (Netherlands), Hμrel Corporation, BioIVT (US), and InSphero (Switzerland) [102]. In addition to the advanced technologies and investments, the collaborations between organ-on-a-chip manufacturing and pharmaceutical companies have contributed to the cost efficiency with respect to drug development and preventing the losses that are incurred as a result of late-stage failures in drug development.

The development of lung-on-a-chip models could largely improve the prospects of more efficient and effective drug toxicity testing and drug development, enable deeper and more human relevant and patient-specific personalized understanding into disease pathophysiology and mechanistic working of the lungs, and extended to the application into toxicity testing. The ability of the lung-on-a-chip to reproduce specific regions of the human respiratory tract and their responses has proved beneficial in the field of toxicity testing. The US Environmental Protection Agency (EPA), in 2018, recognized the valuable use of in vitro model systems—the MucilAir™ model—in refining the risk assessment for the pesticide chlorothalonil and other contact irritants with respect to inhalation toxicity [103]. Although the lung-on-a-chip device is unique, it needs to be developed further into a well-rounded system mimicking the human physiology and pathophysiology to effective drug discovery and development, which could be achieved through collaborative research. Further developments in drug delivery systems that can effectively interface with the lung-on-a-chip model are required to efficiently screen unsuccessful drugs in the early stages of the development process, making it time- and cost-effective and increasing the amount of compounds that can be screened. When there is a better understanding of human diseases, the way for personalized medication can be through the establishment of new therapeutic targets by accessing information

that was previously unavailable due to the limitations faced by traditional methods like animal studies.

The lung-on-a-chip model has the potential to aid in the understanding of human and patient-specific processes in vitro. Further optimization and innovation in this technology could increase the reproducibility while studying complex pulmonary diseases, personalized medicine, drug development, toxicity studies, and uptake of the methods for regulatory use. There is also the possibility of integrating the lung-on-a-chip with other organ-on-a-chip models to develop a 'body-on-a-chip' to understand multi-organ responses in various areas like clinical trials, paving the way for a more human-centric and relevant method for basic and translational research.

References

1. Tortora G, Anagnostakos N (1987) Principles of anatomy and physiology. Harper and Row, New York, p 555
2. Williams PL, Warwick R, Dyson M, Bannister LH (1989) Gray's Anatomy, 37th edn. Churchill Livingstone, Edinburgh, pp 1278–1282
3. Antunes MB, Cohen NA (2007) Mucociliary clearance - a critical upper airway host defense mechanism and methods of assessment. Curr Opin Allergy Clin Immunol 7(1):5–10
4. Walker BR, College NR, Ralston SH, Penman ID (eds) (2014) Davidson's principles and practice of medicine. Elsevier, Amsterdam
5. Stanke F (2015) The contribution of the airway epithelial cell to host defense. Mediat Inflamm 2015:463016
6. Standring S, Borley NR (2008) Gray's anatomy: the anatomical basis of clinical practice, 40th edn. Churchill Livingstone/Elsevier, Edinburgh, pp 992–1000
7. Otto WR (1997) Lung stem cells. Int J Exp Pathol 78:291–310
8. Cui L, Morris A, Ghedin E (2013) The human mycobiome in health and disease. Genome Med 5(7):63
9. Marshall LJ, Oguejiofor W, Willetts RS, Griffiths HR, Devitt A (2014) Developing accurate models of the human airways. J Pharmacol Pharm 67:464–472
10. Breeze RG, Wheeldon EB (1977) The cells of the pulmonary airways. Am Rev Respir Dis 116:705–777
11. Gail DB, Lenfant CJ (1983) Cells of the lung: biology and clinical implications. Am Rev Respir Dis 127:366–387
12. Jeffery PK, Li D (1997) Airway mucosa: secretory cells, mucus and mucin genes. Eur Respir J 10:1655–1662
13. Rogers AV et al (1993) Identification of serous-like cells in the surface epithelium of human bronchioles. Eur Respir J 6:498–504
14. Knight DA, Holgate ST (2003) The airway epithelium: structural and functional properties in health and disease. Respirology 8:432–446
15. Farquhar MG, Palade GE (1963) Junctional complexes in various epithelia. J Cell Biol 17: 375–412
16. Godfrey RW (1997) Human airway epithelial tight junctions. Microsc Res Tech 38:488–499
17. Balcombe JP, Barnard ND, Sandusky C (2004) Laboratory routines cause animal stress. Contemp Top Lab Anim Sci 43:42–51
18. Evans MJ et al (2001) Cellular and molecular characteristics of basal cells in airway epithelium. Exp Lung Res 27:401–415
19. Bienkowski RS, Gotkin MG (1995) Control of collagen deposition in mammalian lung. Proc Soc Exp Biol Med 209:118–140. Royal Society of Medicine

20. Brewster CE et al (1990) Myofibroblasts and subepithelial fibrosis in bronchial asthma. Am J Respir Cell Mol Biol 3:507–511
21. Dunsmore SE, Rannels DE (1996) Extracellular matrix biology in the lung. Am J Phys 270: L3–L27
22. Mutsaers SE et al (1997) Mechanisms of tissue repair: from wound healing to fibrosis. Int J Biochem Cell Biol 29:5–17
23. McAnulty RJ et al (1995) Regulation of fibroblast procollagen production. Transforming growth factor-beta 1 induces prostaglandin E2 but not procollagen synthesis via a pertussis toxin-sensitive G-protein. Biochem J 307(Pt 1):63–68
24. Evans MJ et al (1999) The attenuated fibroblast sheath of the respiratory tract epithelial-mesenchymal trophic unit. Am J Respir Cell Mol Biol 21:655–657
25. Minoo P, King RJ (1994) Epithelial-mesenchymal interactions in lung development. Annu Rev Physiol 56:13–45
26. Crystal RG (2014) Airway basal cells. The "smoking gun" of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 190(12):1355–1362
27. Arvers P (2018) Alcohol consumption and lung damage: dangerous relationships. Rev Mal Respir 35(10):1039–1049
28. Slovinsky WS, Romero F, Sales D, Shaghaghi H, Summer R (2019) The involvement of GM-CSF deficiencies in parallel pathways of pulmonary alveolar proteinosis and the alcoholic lung. Alcohol (Fayetteville, NY) 80:73–79
29. Lutz C, Well A, Novak M (2003) Stereotypic and self-injurious behavior in rhesus macaques: a survey and retrospective analysis of environment and early experience. Am J Primatol 60:1–1
30. Morgan KN, Tromborg CT (2007) Sources of stress in captivity. Appl Anim Behav Sci 102:262–302
31. Suckow MA, Weisbroth SH, Franklin CL (2006) The laboratory rat, 2nd edn. Elsevier Academic Press, Burlington, MA. at 323
32. Flow BL, Jaques JT (1997) Effect of room arrangement and blood sample collection sequence on serum thyroid hormone and cortisol concentrations in cynomolgus macaques (Macaca fascicularis). Contemp Top Lab Anim Sci 36:65–68
33. Baldwin A, Bekoff M (2007) Too stressed to work. New Sci 194:24
34. Akhtar A, Pippin JJ, Sandusky CB (2008) Animal models in spinal cord injury: a review. Rev Neurosci 19:47–60
35. Curry SH (2003) Why have so many drugs with stellar results in laboratory stroke models failed in clinical trials? A theory based on allometric relationships. Ann N Y Acad Sci 993:69–74
36. Dirnagl U (2006) Bench to bedside: the quest for quality in experimental stroke research. J Cereb Blood Flow Metab 26:1465–1478
37. Palfreyman MG, Charles V, Blander J (2002) The importance of using human-based models in gene and drug discovery. Drug Discov World Fall:33–40
38. Harding A (2004) More compounds failing phase I. The Scientist; Sept 13. Available at https://www.the-scientist.com/news-analysis/more-compounds-failing-phase-i-49707. Last accessed 10 Nov 2021
39. Pippin J (2013) Animal research in medical sciences: seeking a convergence of science, medicine, and animal law. South Texas Law Rev 54:469–511
40. Hartung T, Zurlo J (2012) Food for thought... Alternative approaches for medical countermeasures to biological and chemical terrorism and warfare. ALTEX 29:251–260
41. Drazen JM, Finn PW, De Sanctis GT (1999) Mouse models of airwayresponsiveness: physiological basis of observed outcomesand analysis of selected examples using these outcomeindicators. Annu Rev Physiol 61:593–625
42. Gelfand E (2002) Pro: mice are a good model of human airwaydisease. Am J Respir Crit Care Med 166:5–8

43. Tu Y-P, Larsen GL, Irvin CG (1995) Utility of murine systems to study asthma pathogenesis. Eur Respir Rev 29:224–230
44. Gomes RFM, Bates JHT (2002) Geometric determinants of airway resistance in two isomorphic rodent species. Respir Physiol Neurobiol 130:317–325
45. McBride JT (1992) 'Architecture of the tracheobronchial tree'.In treatise on pulmonary toxicology: comparative biology of the Normal lung (edited by: parent RA). CRC Press, Boca Raton, FL, pp 49–61
46. Irvin C, Bates J (2003) Measuring the lung function in the mouse: the challenge of size. Respir Res 4:4
47. Canning BJ, Chou Y (2008) Using Guinea pigs in studies relevant to asthma and COPD. Pulm Pharmacol Ther 21(5):702–720
48. Ochs M, Nyengaard JR, Jung A et al (2004) Randomized controlled trial of domiciliary noninvasive positive pressure ventilation and physical training in severe chronic obstructive pulmonary disease. Am J Respir Crit Care Med 169(1):120–124
49. McMillan SJ, Lloyd CM (2004) Prolonged allergen challenge in mice leads to persistent airway remodelling. Clin Exp Allergy 34(3):497–507
50. Nials AT, Uddin S (2008) Mouse models of allergic asthma: acute and chronic allergen challenge. Dis Model Mech 1(4–5):213–220
51. VivoloAun M, Bonamichi-Santos R (2017) Animal models of asthma: utility and limitations. J Asthma Allergy 10:293–301
52. Gharib SA, Nguyen E, Altemeier WA, Shaffer SA, Doneanu CE, Goodlett DR, Schnapp LM (2010) Of mice and men: comparative proteomicsof bronchoalveolar fluid. Eur Respir J 35:1388–1395
53. Albertine KH, Wiener-Kronish JP, Staub NC (1984) The structure of the parietal pleura and its relationship to pleural liquid dynamics in sheep. Anat Rec 208:401–409
54. Matthay MA, Berthiaume Y, Staub NC (1985) Long-term clearance of liquid and proteinfrom the lungs of unanesthetized sheep. J Appl Physiol 59:928–934
55. Sakuma T, Folkesson HG, Suzuki S, Okaniwa G, Fujimura S, Matthay MA (1997) Beta-adrenergic agonist stimulated alveolar fluid clearancein ex vivo human and rat lungs. Am J Respir Crit Care Med 155:506–512
56. Smedira N, Gates L, Hastings R, Jayr C, Sakuma T, Pittet JF, Matthay MA (1991) Alveolarand lung liquid clearance in anesthetized rabbits. J Appl Physiol 70:1827–1835
57. Proudfoot AG, McAuley DF, Griffiths MJD, Hind M (2011) Human models of acute lung injury. Dis Models Mech 4:145–153
58. Huh D, Matthews B, Mammoto A, Montoya-Zavala M, Hsin H, Ingber D (2010) Reconstituting organ-level lung functions on a Chip. Science 328(5986):1662–1668
59. Napierska D, Thomassen L, Rabolli V, Lison D, Gonzalez L, Kisrch-Volders M, Martens J, Hoet P (2019) Size-dependent cytotoxicity of monodisperse silica nanoparticles in human endothelial cells. Small 5(7):846–853
60. Quirós-Solano WF, Gaio N, Stassen OMJA, Arik YB, Silvestri C, Van Engeland NCA, Van der Meer A, Passier R, Sahlgren CM, Bouten CVC, van den Berg A, Dekker R, Sarro PM (2018) Microfabricated tuneable and transferable porous PDMS membranes for organs-on-chips. Sci Rep 8:13524
61. Paul A, Stührenberg M, Chen S, Rhee D, Lee W-K, Odom TW, Heilshorn SC, Enejder A (2017) Micros- and nan-patterned elastin-like polypeptide hydrogels for stem cell culture. Soft Matter 3:5665–5675
62. Wise SG, Liu H, Yeo GC, Michael PL, Chan AHP, Ngo AKY, Bilek MMM, Bao S, Weiss AS (2016) Blended polyurethane and tropoelastin as a novel class of biologically interactive elastomer. Tissue Eng Part A 22:524–533
63. Murphy WL, TC MD, Engler AJ (2014) Materials as stem cell regulators. Nat Mater 13:547–557
64. Engler AJ, Sen S, Sweeney HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. Cell 126:677–689

65. Schürch D, Vanhecke D, Clift MJD, Raemy D, de Aberasturi DJ, Parak WJ, Gehr P, Petri-Fink A, Rothen-Rutishauser B (2014) Modeling nanoparticle-alveolar epithelial cell interactions under breathing conditions using captive bubble surfactometry. Langmuir 30:4924–4932
66. Bauer A, Gu L, Kwee B, Li WA, Dellacherie M, Celiz AD, Mooney DJ (2017) Hydrogel substrate stress-relaxation regulates the spreading and proliferation of mouse myoblasts. Acta Biomater 62:82–90
67. Darnell M, Young S, Gu L, Shah N, Lippens E, Weaver J, Duda G, Mooney D (2017) Substrate stress-relaxation regulates scaffold remodeling and bone formation in vivo. Adv Healthc Mater 6:1601185
68. Lv H, Li L, Sun M, Zhang Y, Chen L, Rong Y, Li Y (2015) Mechanism of regulation of stem cell differentiation by matrix stiffness. Stem Cell Res Ther 6:103–113
69. HinzB. (2012) Mechanical aspects of lung fibrosis. Proc Am Thorac Soc 9:137–147
70. Johnston ID, McCluskey DK, Tan CKL, Tracey MC (2014) Mechanical characterization of bulk Sylgard 184 for microfluidics and microengineering. J Micromech Microeng 24:035017
71. Deguchi S, Hotta J, Yokoyama S, Matsui TS (2015) Viscoelastic and optical properties of four different PDMS polymers. J Micromech Microeng 25:097002
72. Pasman T, Grijpma D, Stamatialis D, Poot A (2018) Flat and micro structured polymeric membranes in organs-on-chips. J R Soc Interface 15:20180351
73. Bajaj P, Harris JF, Huang JH, Nath P, Iyer R (2016) Advances and challenges in recapitulating human pulmonary systems: at the cusp of biology and materials. ACS Biomater Sci Eng 2 (4):473–488
74. Doryab A, Amoabediny G, Salehi-Najafabadi A (2016) Advances in pulmonary therapy and drug development: lung tissue engineering to lung-on-a-chip. Biotechnol Adv 34(5):588–596
75. Huh D, Fujioka H, Tung YC et al (2007) Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems. Proc Natl Acad Sci U S A 104(48):18886–18891
76. Douville NJ, Zamankhan P, Tung YC et al (2011) Combination of fluid and solid mechanical stresses contribute to cell death and detachment in a microfluidic alveolar model. Lab Chip 11 (4):609–619
77. Tavana H, Zamankhan P, Christensen PJ, Grotberg JB, Takayama S (2011) Epithelium damage and protection during reopening of occluded airways in a physiologic microfluidic pulmonary airway model. Biomed Microdev 13(4):731–742
78. Moraes C, Mehta G, Lesher-Perez SC, Takayama S (2012) Organs-on-a-chip: a focus on compartmentalized microdevices. Ann Biomed Eng 40(6):1211–1227
79. Yu T, Guo Z, Fan H et al (2016) Cancer-associated fibroblasts promote non-small cell lung cancer cell invasion by upregulation of glucose-regulated protein 78 (GRP78) expression in an integrated bionic microfluidic device. Oncotarget 7(18):25593–25603
80. Huh D, Leslie DC, Matthews BD et al (2012) A human disease model of drug toxicity–induced pulmonary edema in a lung-on-a-chip microdevice. Sci Transl Med 4(159):159ra147
81. Benam KH, Villenave R, Lucchesi C, Varone A, Hubeau C, Lee HH et al (2016) Small airway-on-a-chip enables analysis of human lung inflammation and drug responses in vitro. Nat Methods 13:151–157
82. Benam KH, Mazur M, Choe Y, Ferrante TC, Novak R, Ingber DE (2017) Human lung small airway-on-a-chip protocol. Methods Mol Biol 1612:345–365
83. Benam KH, Novak R, Nawroth J, Hirano-Kobayashi M, Ferrante TC, Choe Y et al (2016) Matched-comparative modeling of normal and diseased human airway responses using a microengineered breathing lung chip. Cell Syst 3:456–466.e4
84. https://wyss.harvard.edu/news/modeling-copd-and-asthma/. Last Accessed 10 Nov 2021
85. Plebani R, Potla R, Soong M, Bai H, Izadifar Z, Jiang A, Travis RN, Belgur C, Cartwright MJ, Prantil-Baun R, Jolly P, Giplin SE, Romano M, Ingber DE. Modeling pulmonary cystic fibrosis in a human lung airway-on-a-chip. MedRxiv. https://doi.org/10.1101/2021.07.15.21260407

86. Alikadic S, Horak F, Frischer T, Nachbaur E, Renner S, Gruber S (2011) Ciliary beat frequency in nasal and bronchial epithelial cells in patients with cystic fibrosis. Eur Respir J 38:4550
87. Emerson J, Rosenfeld M, McNamara S, Ramsey B, Gibson RL (2002) Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. Pediatr Pulmonol 34:91–100
88. Isopi E, Mattoscio D, Codagnone M, Mari VC, Lamolinara A, Patruno S et al (2020) Resolvin D1 reduces lung infection and inflammation activating resolution in cystic fibrosis. Front Immunol 11:581
89. Asmani M, Velumani S, Li Y et al (2018) Fibrotic microtissue array to predict anti-fibrosis drug efficacy. Nat Commun 9(1):2066
90. Zamprogno P, Wüthrich S, Achenbach S, Thoma G, Stucki JD, Hobi N, Schneider-Daum N, Lehr C-M, Huwer H, Geiser T, Schmid RA, Guenat OT (2021) Second-generation lung-on-a-chip with an array of stretchable alveoli made with a biological membrane. Commun Biol 4:168
91. Yang X, Li K, Zhang X et al (2018) Nanofiber membrane supported lung-on-a-chip microdevice for anti-cancer drug testing. Lab Chip 18(3):486–495
92. Xu Z, Gao Y, Hao Y et al (2013) Application of a microfluidic chip-based 3D co-culture to test drug sensitivity for individualized treatment of lung cancer. Biomaterials 34(16):4109–4117
93. Si L, Bai H, Rodas M, Cao W, Oh CY, Jiang A, Nurani A, Zhu DY, Goyal G, Gilpin SE, Prantil-Baun R, Ingber DE (2020) Human organs-on-chips as tools for repurposing approved drugs as potential influenza and COVID19 therapeutics in viral pandemics. BioRxiv. https://doi.org/10.1101/2020.04.13.039917
94. Zhang B, Radisic M (2017) Organ-on-a-chip devices advance to market. Lab Chip 17:2395–2420
95. Rayat C, Joshi K, Sakhuja V et al (2005) Glomerular basement membrane thickness in normal adults and its application to the diagnosis of thin basement membrane disease: an Indian study. Indian J Pathol Microbiol 48:453–458
96. Esch EW, Bahinski A, Huh D (2015) Organs-on-chips at the frontiers of drug discovery. Nat Rev Drug Discov 14:248–260
97. Van den Berg A, Mummery CL, Passier R et al (2019) Personalised organs-on-chips: functional testing for precision medicine. Lab Chip 19:198–205
98. Vatine GD, Barrile R, Workman MJ et al (2019) Human iPSC-derived blood-brain barrier chips enable disease modeling and personalized medicine applications. Cell Stem Cell 24:995–1005.e6
99. Gkatzis K, Taghizadeh S, Huh D et al (2018) Use of 3D organoids and lung-on-a-chip methods to study lung development, regeneration and disease. Eur Respir J 52:1800876
100. Nawroth JC, Barrile R, Conegliano D et al (2018) Stem cellbased lung-on-chips: the best of both worlds? Adv Drug Deliv Rev 140:12–32
101. Schilders KAA, Eenjes E, van Riet S et al (2016) Regeneration of the lung: lung stem cells and the development of lung mimicking devices. Respir Res 17:44
102. https://www.medgadget.com/2021/09/organ-on-a-chip-market-size-is-projected-to-reach-usd-1020-million-at-53-cagr-by-2027.html. Last accessed 10 Nov 2021
103. EPA (2018) Evaluation of a proposed approach to refine inhalation risk assessment for point of contact toxicity: a case study using a new approach methodology (NAM). Available online at: https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2019/september/bcgnd-1-epa_case_study.pdf

Brain-on-a-Chip

21

Subhadra Nandi, Satyajit Ghosh, Shubham Garg, Ankan Sarkar, and Surajit Ghosh

Abstract

Unravelling the microenvironment of the human brain to investigate the different pathophysiological conditions is a pivotal task. The demarcation of the central nervous system from the peripheral nervous system makes the human brain developmental process a more protracted one. Increasing number of various neurodegenerative diseases causes a major challenge to the management of global public health. Although traditional in vivo animal models and in vitro cell culture models facilitate the evaluation of the structural and functional aspects of the brain, they have certain limitations too. This calls for an innovative approach in the shape of Microfluidics Technology that can offer a single platform to culture neurons in a quasi-physiological confined microenvironment, to reconstitute neural framework under spatiotemporal regulation and external stimulation for modelling of neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and more followed by high-throughput analysis of effective drug delivery systems. In this chapter, we have highlighted the various structural and functional aspects of the human brain by the reconstitution of the central nervous system and the peripheral nervous system on chip. We have thereby highlighted the neurological disorders that are associated with the malfunctioning of both the nervous systems succeeded by high-throughput analysis of drugs on chip that have been screened till date. Therefore, these lab-on-chip technologies hold promises for mimicking the complex brain microenvironment imparting novel platform for disease modelling and drug screening.

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Keywords

Neural cytoarchitecture · Brain microenvironment · Neurodegenerative disease · Microfluidics Technology · Drug screening

21.1 Introduction

The most vital component of the human body is the brain and early human brain development is a complex phenomenon wherein reconstitution of the distinctive architecture of the prevailing networks and the intracellular interactions for the proper functioning of the brain is a daunting task. The development of the human brain is a prolonged mechanism that initially starts with the formation of the neural plate, which then folds and gives rise to a zipper-like structure called the neural tube. The central nervous system (comprising of the brain and spinal cord) is formed from the inner cells of the neural tube, whereas the peripheral nervous system (comprising nerves outside the brain and spinal cord) is formed by the outer cells. This whole event of the formation of distinct brain structures is called neurulation. Once neurulation is complete, the cells lining the innermost part of the neural tube (known as the ventricular zone) proliferate and give rise to a marginal zone that consists of the axons and dendrites [1]. Next, radial migration includes the migration of pyramidal neurons (that send signals to different cortical layers as well as other parts of the brain) and glia (the non-neuronal cells of the brain) to target destinations [1] where the neurons either differentiate into a mature neuron comprising the complete axon (whose development is guided by growth cones) and dendrites or are withdrawn through apoptosis [2]. The point of contact between brain cells is established by synapse [3] through a process called synaptogenesis, which is followed by the final step of myelination (in which the axons are insulated to transmit electrical signals), which completes the brain development [4]. To investigate the structural and functional aspects of the brain and to evaluate any disorders related to its malfunction, exploration of the subcellular level is very crucial that provides deep insights into the neural network development and cytoarchitecture (Fig. 21.1).

Although traditional in vivo animal models and in vitro cell culture models are used to evaluate the structural and functional aspects of the brain, they often have some associated drawbacks too. In spite of their abilities to assess the safety, toxicity, and efficacy of potential drug molecules, in vivo animal models lead to genetic heterogeneity and loss of the immune system and cause obstacles in pharmacokinetic predictions. Moreover, the ethical concerns are also high in comparison to any other models [5]. On the other hand, 2D cell culture models require a higher amount of samples and reagents and they fail to mimic mechanical properties, fluid flow, and other physiological conditions of human brain tissue. Current 3D cell culture models like spheroids cells are capable of establishing contacts [6], but fail to fully recapitulate interactions between cells of different types [7]. The organoid culture, which is a better biomimetic tissue model [8], exhibits low uniformity and reproducibility [9]

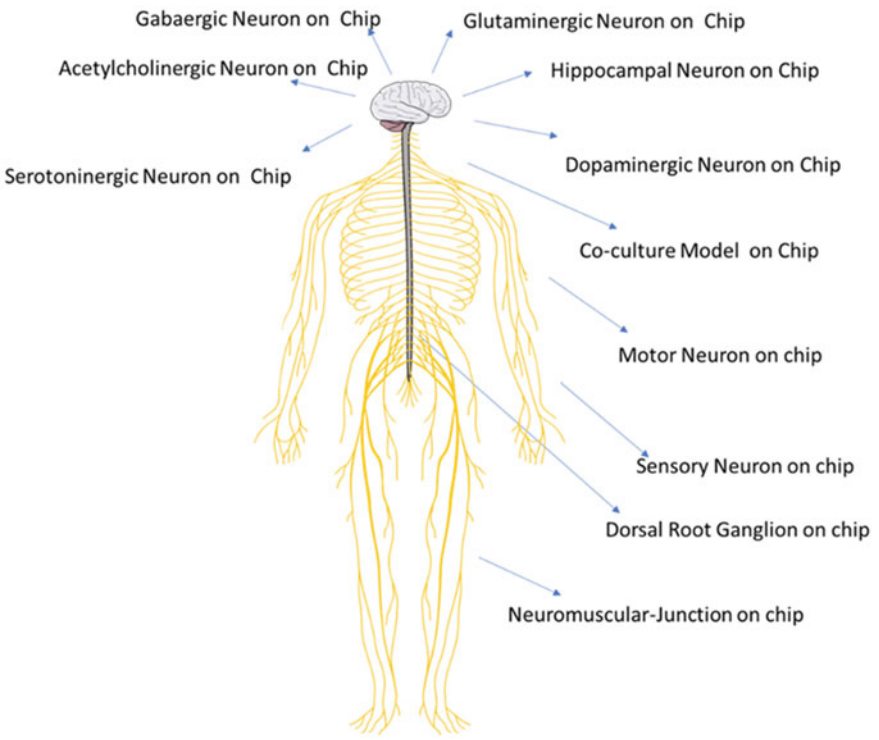

Fig. 21.1 Multifaceted models of the central nervous system and peripheral nervous system-based microfluidics device

and features like shear stress caused by blood flow and stroma are often not considered [10]. Hence, all these hurdles give rise to the inception of the concept of miniaturization in the form of Microfluidics Technology.

Microfluidics is all about studying flows with characteristic length scales of the order of microns. Microfluidics Technology deals with the exploitation of nanolitre (10^{-9} to 10^{-18} L) volumes of fluids to study their behaviour as it comprises of channels in the order of micrometre range (generally tens to a hundred micrometres) [11]. It becomes easier to mimic the interaction of the brain cells such as intercellular interactions between various cell types like microglia, oligodendrocytes, pericytes, endothelial cells, etc. in a single platform. The two most important parameters such as stiffness [12] and viscoelasticity [13] can be efficiently analysed in the microfluidic device. The microfluidic system provides a much facile platform to mimic the CNS-like microenvironment more appropriately. The continuous flow of culture medium and removal of waste help to maintain nervous tissue homeostatic balance on the chip. This property is advantageous for deciphering the event of neural tube formation during early brain development as was depicted by Uzel et al., who studied the effect of a concentration gradient of retinoic acid and sonic hedgehog (SHH) on the differentiation of neural stem cells [14]. Brown et al.

delineated a much closer resemblance of the cells of CNS through their vertically made compartmentalized culture system where they cultured endothelial cells in the lower chamber that was distinct from the upper chamber containing layers of pericytes and astrocytes as well as neurons by a semi-permeable membrane [15]. The existence of a blood-brain barrier (BBB) is the most crucial component of the CNS that restricts harmful substances from entering into the brain, but also hinders the efficient delivery of drugs in the case of neurological disorders. Researchers have mimicked the BBB to screen the effects of drugs such as cimetidine and doxorubicin [16] and also to examine neuro-inflammation on chips by adding certain pro-inflammatory cytokines to activate endothelial cells to induce the flow of leukocytes through the microchannels [17].

Not only CNS, but researchers have also tried to reconstitute the human PNS on the microfluidic device to study neurological disorders. For example, Hyung et al. created a physiologically relevant three-dimensional platform to recapitulate demyelinating disorders by the co-culture of primary Schwann cells and motor neurons using reproducible hydrogel patterning [18]. Therefore, the above studies reveal the importance and necessity of an all-in-one miniaturized platform to unravel the mysteries prevailing in the human brain microenvironment followed by generation of neurodegenerative disease models and subsequent drug screening (Fig. 21.2).

21.2 Reconstitution of the Central Nervous System on Chip

21.2.1 Dopaminergic Neuron on Chip

Dopamine (DA) as a neurotransmitter was first identified in the late 1950s by ArvidCarlsson; earlier, DA was considered a metabolic precursor for another neurotransmitter, norepinephrine [19, 20]. Although Dopaminergic neurons (DN) constitute only 1% of the total neuron in the brain, it is one of the most important and intensively studied neurotransmitters. Due to its essential involvement in a vast array of normal brain functions, DN is also considered behaviourally robust neurons. Midbrain is the primary source of DN in the CNS of the mammalian brain, located in the substantial nigra pars compacta (SNc) and Ventral tegmental area (VTA) [19]. Neurons of SNc and VTA communicate with the encounter signal in two modes, “tonic” and “phasic.” In their phasic mode, the concentration of DA changes sharply due to a change in the firing potential of DA neurons, whereas, in tonic mode, the concentration of DA remains in a steady state [21]. Parkinson’s disease is the most common disease associated with the dysfunctioning of DN from Substantia Nigra; the result of PD is loss of DN, which ultimately leads to a decline in DA levels; as a result, most of the body motor functions are affected, which ultimately leads to severe movement disorders, tremors, and postural instability. Current methods (ELISA) for examining the DN dysfunction depend on measuring the level of DA uptake by DN [22]. However, there are several challenges associated with current methods like they suffer from low throughput and are also not suitable for laboratory-based screening.

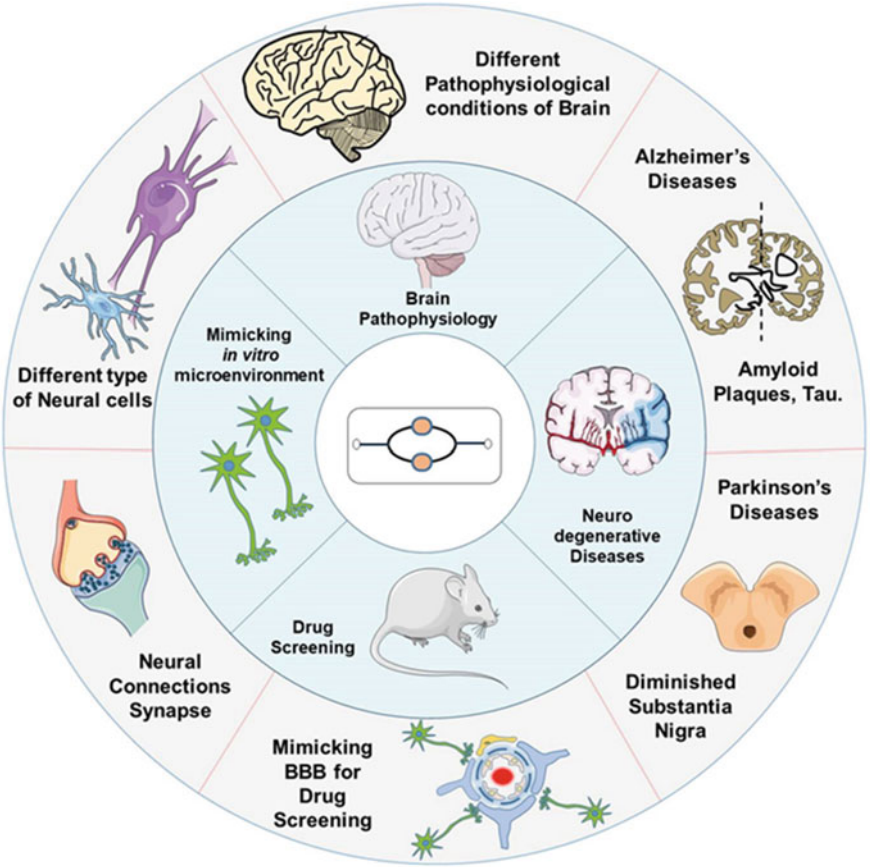

Fig. 21.2 Schematic illustration of a Microfluidics Platform mimicking brain microenvironment, brain pathophysiological conditions, and subsequent high-throughput drug screening

Methods that could rely on sensitive and selective detection of DA concentration are of utmost priority for the clinical relevance of PD. In this manner, a microfluidics platform could provide a possible solution, like miniaturization of sensing technology could help detect neurotransmitters like DA, which are found in concentrations of as low as 1 nM [23]. In the recent work for the first time, Senel M. et al. developed a microfluidic platform with a meager working volume of 2.4 μL with high accuracy and a precise detection limit of 0.1 nmol of DA. One of the main advantages of using this kind of device is that they can even detect the DA level from the scarcely available samples such as human CSF [23].

Stem cell-based therapeutics can be an ideal candidate for the treatment of PD. There have been already some studies in clinical trials where stem cells are being used to cure PD [24]. Another promising therapy would be to use stem cells along with some other therapeutics like levopoda. Microfluidics has been developed

for a myriad of biological applications and has the intrinsic capabilities to be used in regenerative medicine. The microfluidic prototype is also used to differentiate stem cells into dopaminergic neurons successfully [25]. A recent study by Worp H. et al. used a phase-guided three-dimensional microfluidic device to generate active DN from induced pluripotent stem cells [26]. The neurons formed were also immunoreactive for hallmark enzyme of DA synthesis, i.e. tyrosine hydroxylase. This kind of device can play a pivotal role in developing personalized drug delivery for treating PD. Though animal models have been frequently used as a closet approximation of the complex mammalian (human) brain physiology, the experimental data obtained from animal models remain controversial in clinical trials [27]. The microchips can potentially replace the animal models, provide more meaningful insight for drug development, and accurately mimic complex brain physiology. These devices can be used to mimic the functional connectivity between the specific brain regions and different body parts.

21.2.2 Hippocampal Neuron on Chip

Hippocampus is located deep into the inner folds of the bottom middle section of the brain's temporal lobe. Due to its structural similarity with the seahorse, it gets its name as the hippocampus. The hippocampus is also considered a gateway for short memories to be stored in a long-term memory bank. Recent studies on this brain region have advanced our understanding of its distinct role in memory storage and retrieval. Due to its clinical relevance, this is the most extensively studied part of the brain. Loss of memory/Dementia is the first symptom associated with the damage of the brain's hippocampal region. Worldwide, around 50 million people have had dementia, and this is expected to reach 78 million within the next 10 years and to 139 million by the year 2050 [28].

Alzheimer's Disease (AD) alone can contribute to almost 60–70% of dementia cases. It is progressive, irreversible damage to brain memory cells, which ultimately leads to dementia. Abnormal deposition of amyloid plaques, phosphorylation of tau protein, and hyperactivation of glial cells are the three characteristic pillars, which mark the onset of diseases. Due to the limitation of current 2D models, there is a growing need for alternative in vitro models to understand the complete etiology of diseased conditions. Moreover, except few models, recent studies show that most animal models of AD cannot wholly mimic all the pathophysiological conditions in humans [29].

Even though significant efforts have been made for the treatment of neurodegenerative diseases, the fundamental mechanism behind these neurodegenerative diseases is still unclear. Developing a realistic model of AD has been particularly challenging due to its complex pathogenesis. Reconstituting critical features of the brain on a microfluidics chip-based 3D Neurosphere can be the alternative seat of action for early diagnosis, drug screening, and cure of neurodegenerative diseases like AD (Table 21.1). Park et al. in the year 2015 developed an in vivo mimicking 3D microfluidic chip to study the effect of interstitial flow on neurospheroid growth

Table 21.1 Microfluidic-based drug screening of various CNS and PNS originating diseases

Sr. no.	Drugs	Disease	Description
1.	Necrostatin [30]	ALS	It is a receptor interacting serine/threonine kinase 1 (RIPK1) inhibitor that was found to reduce muscle contraction in SOD1 astrocytes-motor neuron co-culture along with swerving of the denervated myofiber.
2.	Pridopidine [31]	ALS	Screening of pridopidine on-chip exhibits formation of NMJs, cluster of AChR at NMJ, and improved axonal transport.
3.	Bosutinib-Rapamycin [32]	ALS	Cotreatment of both on ALS microfluidic device shows protection on motor domain and reinstate muscle function.
4.	Spinraza [33]	SMA	First approved drug for SMA. Lipid nanoparticle delivery of SPINRAZA on a microfluidic platform.
5.	Rapamycin-Riluzole [34]	ALS	The study conducted demonstrates autophagy-induced TDP-43 clearance in ALS.
6.	Tacrine [35]	AD	Kinetic study of tacrine by droplet-based microfluidic system for drug screening.
7.	Pramipexole [36]	PD	Efficacy study and thrashing force measurement of pramipexole PDMS microfluidic device.

[37]. Though there have been studies on the effect of flow on 2D neural cultures, there are no reported studies on the effect of flow on 3D neurospheroid models. These devices are beneficial in studying the role of nutrient delivery and drug screening and clearance.

In addition to studies involving one such study, Oliveria and her co-workers developed a cost-effective and straightforward microfluidics-based platform for the early diagnosis of AD using a blood biomarker called "A Disinterring and Metalloprotease 10" ($ADAM_{10}$) at as low as 0.35 fg/mL concentration in serum [38]. One of the main significant findings to emerge from these studies is that a 3D chip has excellent potential as an in vitro brain model for understanding diseases' pathophysiology and drug discovery. These in vivo microenvironments created by 3D chips will indeed have great potential to be used as a model to mimic complex brain pathogenesis.

21.2.3 Cholinergic, Gabaergic, Glutaminergic, and Serotoninergic Neuron on Chip

Many research groups are working on culturing different types of neurons in a microfluidic chip and mimicking the actual brain environment in normal and diseased conditions. Fantuzzo et al. have developed a multi-compartmentalized (Four outer chambers were connected to a central chamber through a circular array of microchannels) microfluidics platform capable of housing multiple neuronal subtypes. They demonstrated the generation of three different neuronal populations,

Dopaminergic, glutamatergic, and GABAergic neurons, from iPS. This device was unique and appropriate for patch-clamp apparatus, which enables the precise recording of neuronal activity. Neurons within the centre compartment can receive inputs from multiple side chambers; this property makes the device unique for the development of the brain mimetic neurocircuit model [39]. Maoz et al. have developed an innovative and sophisticated platform of three interconnected microfluidic systems. The platform is composed of two blood-brain barrier (BBB) chips and a brain-on-chip in between, which reconstitute the brain parenchyma and the influx/efflux across the BBB at the same time. For the brain chip development, human neural stem cells (~60% glial cells and 40% of dopaminergic, glutamatergic, serotonergic, and GABAergic neurons) and astrocytes were cultured on the laminin and poly-l-lysine-coated surface of the lower compartment, whereas the BBB chips were developed by culturing a monolayer of primary human brain microvascular endothelial cells (hBMVECs) and astrocytes and pericytes at the vascular chamber and at the perivascular chamber, respectively [40]. Tourovskaia et al. have developed a PDMS-based microfluidic device to focally deliver agrin to presynaptic acetylcholine receptor (AChR) clusters in micropatterned myotube cultures. From this study, they have successfully shown that focal agrin stimulus can play a key stabilizing role in the aggregation of AChRs at the early stages of synapse formation [41]. This microfluidic model can, therefore, be used for diverse applications, from studying brain pharmacokinetics to studying the efficacy and toxicity of CNS-targeted drugs.

21.2.4 Co-culture Study in Microfluidics Platform

Microfluidics-based cell mimicking nervous systems offer physiologically relevant models for studying disease and development. Co-culture of neurons with other cells of the nervous system in a 3D microfluidic platform will mimic appropriate cellular ecosystems, which will help in a better understanding of normal and abnormal conditions related to the nervous system [42]. With the advancement of this field, many devices have been fabricated to co-culture neurons with non-neuronal cells, such as glia [43] and oligodendrocytes [44]. Majumdar et al. developed a device to culture neurons and glia, which gives the advantage of high-resolution microscopy and high-efficiency transfection of neurons [45]. Park et al. fabricated a compartmentalized co-culture platform that can be used in axon myelination study. The platform is a combination of a soma compartment and axon/glia compartment, which is connected through arrays of axon-guiding microchannels [46]. Two novel set-ups, one vertically layered and a four-chamber, have been developed by Shi et al. for co-culturing CNS neurons and glia. This device facilitated the culture of glia with neurons in close proximity and gave them an opportunity to visualize neuronal interactions, like synapses development. They have reported that neuron-glia co-cultures produced elevated levels of soluble factors compared to that secreted by individual neuron or glia cultures. Using this model, they have concluded that communication between neurons and glia is critical for the formation and stability of synapses [47].

Adriani et al. mimicked the physiological characteristics of the blood-brain barrier (BBB) by utilizing a 3D triple co-culture microfluidic system using human umbilical vein endothelial cells (HUVEC) together with primary rat astrocytes and neurons. This device enables the study of BBB permeability and neuron functionality [48]. Yong et al. have demonstrated a device that can be used to study axon degeneration. They have used cases of nucleation and trophic deprivation to reproduce their effect in the microfluidics-based axon degeneration model [49]. Studies have indicated that researchers have got a significant amount of success in generating disease models using a co-culture-based microfluidics platform, which can be helpful in drug screening and to get a clear understanding of the disease [50–56]. Researchers have also generated neuromuscular junction-on chip models to study different aspects of neuron and muscle cell interaction. Takeuchi et al. have co-cultured superior cervical ganglion (SCG) neurons and ventricular myocytes (VMs) derived from a rat on a microelectrode array (MEA) substrate to study network interactions between sympathetic neurons and cardiomyocytes [57]. Neto et al. have established and characterized a co-culture model using sensory neurons and osteoblasts to mimic the in vivo condition [58]. Lei et al. have come up with a novel platform to study the dynamic interaction between neurons and cancer cells. They have shown that nerve bundles provide biophysical support for cancer cells and guide their directional migration. This model is also suitable for in vitro screening of molecules that can block cancer cell migration along neurites [59].

The advancement of this field is largely due to advancements in manufacturing and improvements in semi-transparent, biocompatible electrode arrays for neuronal studies. In spite of its advantages, the major concern of this field is the unavailability of human-derived neural cells for developing clinically relevant models. Induced pluripotent stem cell (iPSC)-derived neurospheres, organoids, and neurons will provide an opportunity to avoid the disadvantages related to this field.

21.3 Reconstitution of the Peripheral Nervous System on Chip

21.3.1 Sensory Neuron on Chip

The sensory neuron is the part of the peripheral nervous system, which carries messages from the environment via receptors in the skin, muscles, and other external or internal sensory organs to the spinal cord then to the brain. A sensory system, also termed primary afferents, is derived from the neural crest and consists of pseudo bipolar sensory neurons, neural pathways, and brain parts involved in sensory perception [60, 61]. Some progenitor cells derived from the neural crest are bipotent; they can generate sensory neurons and the satellite glia. The satellite glia plays a role in envelope formation and supports the neurons housed in sensory ganglia [62]. The sheath formed by satellite glia contains interspaced gaps that work as a site for the penetration of dye tracers, neurotransmitters, and other macromolecules and electrical coupling between satellite glia, facilitating coordination of function mediated by gap junctions [63]. In contrast to central nervous system (CNS) neurons, the sensory

neuron cell bodies lack a blood-brain barrier. Due to this structural feature, the sensory neurons are at high risk of exposure to inflammatory mediators, systemic drugs, or toxins [64]. The sensory nervous system has two-component, the visceral sensory system and the somatosensory system. The cell bodies of the visceral sensory system are located in bilateral ganglia, including dorsal root ganglion, the geniculate ganglia of the facial nerve, distal glossopharyngeal ganglia (petrosal ganglia), and distal vagal ganglia of cranial nerves (Cr.N.) VII, IX, and X, respectively. Visceral sensory fibres are mechanosensitive or chemosensitive, A-beta, C, and A-delta fibres. A-beta mechanosensitive afferents are found in the trachea and airways and cough reflexes and altered breathing patterns [65, 66]. The somatosensory system conveys sensations of touch, pain, and position of the extremities. Somatic afferents of the head arise from cell bodies found primarily within the trigeminal ganglia, whose peripheral axons form the trigeminal nerve (Cr.N. V). Trigeminal neurons mediate touch, pain, and temperature sensation of the face and forehead and the oral and nasal mucosa. Classic descriptions of somatosensory neurons often ascribe C and A-delta fibres to nociception and A-beta fibres to touch. There are also A-alpha and A-beta proprioceptors that innervate muscle and joints to encode the sensation of body position in space [60]. Due to this complexity, merely plating peripheral neurons on planar surfaces does not allow for recreation of this morphology. Conventionally, two-dimensional (2D) culture models and animal models have been used to mimic the complexity of the brain, but in both cases, some demerits result in over or underestimated cellular behaviours and drug responses. In recent years, microfluidics-based system is immerging as a tool to mimic different complex human architectures. The main factors that remain as hurdles in the path of development of the brain mimetic system are as follows: (1) The brain's distinct microenvironment, mimicking the two important parameters stiffness [67] and viscoelasticity [68] of the brain, is very difficult in plastic and glass substrate-based culture system; (2) Cellular architecture; (3) Multiple cell types; (4) Different functionalities of different brain regions; and (5) the brain contains different subunits that have a different role in homeostasis and disease and have a significant role in drug delivery. Towards this endeavour to mimic peripheral sensory neural system and disease state in microfluidics chip, Sidhu et al. in 2011 have developed polydimethylsiloxane (PDMS)-based "H" shaped microfluidic system to distinguish the two populations of dorsal root ganglia (DRGs) by differential staining using calcein-AM and orange cell tracker. Johnson et al. and Sakai et al. have used 3D printed microchannels and shown the channel-guided outgrowth of DRG axon and have also shown successful induction of myelination and Schwann cell proliferation by co-culturing Schwann cells and DRG-derived neurons [69, 70]. Anderson et al. have used laminin derivatized capillary alginate gel (Capgel™) to culture Embryonic dorsal root ganglion (DRG) explants and shown that the microchannels have improved nerve growth through the gel and they were successful in mimicking normal nerve development, including Schwann cell myelination [71]. Sharma et al. have developed an in vitro biomimetic microfluidic model of all-human peripheral nerve tissue that shows robust neurite outgrowth (~5 mm) and myelination of hNs by primary human Schwann cells (~5%), and they have also successfully evaluated

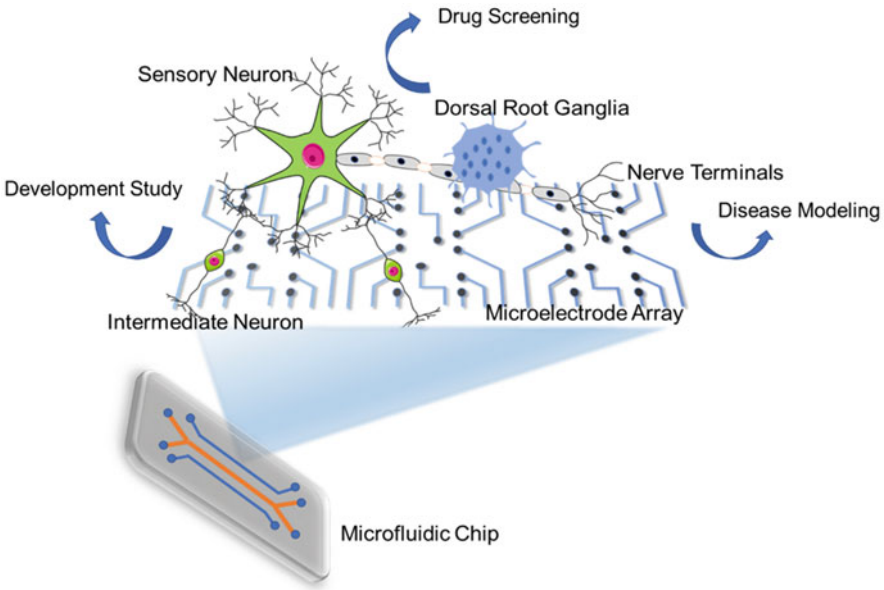

Fig. 21.3 Mimicking Sensory Nervous System on Chip. These neural sensory constructs can integrate a variety of neural subtypes, which can be used to study sensory neuron development and related diseases

nerve conduction velocity (0.13–0.28 m/s) using the same [72]. Apart from modelling, many research teams are working on mimicking sensory neuron-related diseases. Tsantoulas et al. have fabricated a microfluidic device to investigate the changes in axonal excitability following sensitization by nerve growth factor (NGF), injury by axotomy, and interaction with keratinocytes [73]. To investigate the effect of axotomy on synaptic transmission between dorsal root ganglia neurons and dorsal horn neurons, Vysokov et al. reconstructed the first pain synapse in a novel microfluidic-based compartmentalized cell culture system, which recapitulates the connectivity of peripheral pain signalling. Using the same platform, Vysokov et al. screened A803467, ProTXII, and AHTTX and concluded that in cases of neuropathic pain, administration of NaV1.7 and NaV1.8 blockers in conjunction with blockers of NaV1.6 could be highly effective in reducing pain phenotype [74]. Given the aforementioned structural and functional benefits of modelling the development, physiology, and disease condition of sensory neural tissues in a microfluidic device, it is clear that this system can be the supreme and reliable option in developing brain mimetics (Fig. 21.3).

21.3.2 Motor Neuron on Chip

Motor neurons are neuronal cell body which is primarily located in the central nervous system and is classified into upper and lower motor neuron. Upper motor neurons (UMNs) are primarily situated in motor strips of cortical area confined to glutaminergic connections that target within CNS, whereas lower motor neurons (LMNs) are located in the spinal cord's ventral horn and the brain stem extends itself outside the CNS and receives parasympathetic inputs like a cholinergic release [75]. LMNs are extensively studied as their dysfunction leads to the birth of several pathologies. Alpha and gamma motor neurons of LMNs are innervated to extrafusal and intrafusal fibers of skeletal muscle, respectively, and also beta fibers are innervated to both the fibers. α-MNshelp in muscle spindle contraction and sensory inputs were received by β-MNs [76, 77].

21.3.2.1 Reconstitution of Motor Neurons, Axonal Outgrowth, and Formation of the Neuromuscular Junction on a Microfluidic Platform

The microfluidic platform serves as a remarkable substitute for traditional cell culture and potential to eliminate expensive, time-consuming in vivo screening by providing dynamic cellular conditions. It amalgamates the principle of engineering and biological sciences by incorporating biophysical and biochemical cues to form a cellular microenvironment. Compartmentalization by microfluidic devices provides intercellular interaction, oxygen permeability, and biophysiological similarity. Axonal transportation of various substitutes in motor neurons is needed for proper functionality like buffering of calcium, mitochondrial transfer, and initiating signaling cascades. Microfluidic compartmentalization of axon and allied bodies renders spatial arrangement and separation to study cellular processes [78]. Axons extending from the soma of neuron form synapses that are the basis of neuron-to-neuron communication and are crucial for the conduction of electrical and chemical activity. Transmission of electrical signals towards other neurons is crucial for maintaining neuronal homeostasis. Axonal damage and dysfunction give rise to various neuronal pathologies.

Initially, compartmentalization was done by Campenot with a petridish three-chamber culture system separated by a Teflon divider attached to the base by silicone grease. Upon application of NGF to distal chambers rather than the central chamber allows the neurite extension to the distal ones, permitting separation of neuronal soma and axons through the greasy layers [79]. Later, Taylor et al. created microfluidic PDMS channels with four reservoirs connected by microgrooves. Neurons from rats were added to the somal reservoir, which then by capillary action through microchannels moves towards the axonal compartments. Pure and crude axons can be isolated from the axonal compartment for detection of axonal mRNA in neurons also as a model for axonal injury and degeneration [44]. Altman et al. cultured motor neurons extracted from the excluded dorsal horns of HB9:GFP embryonic spinal cord. PDMS multichannel chips were prepared and MNs were cultured, which demonstrated local functions of motor neurons with transportation

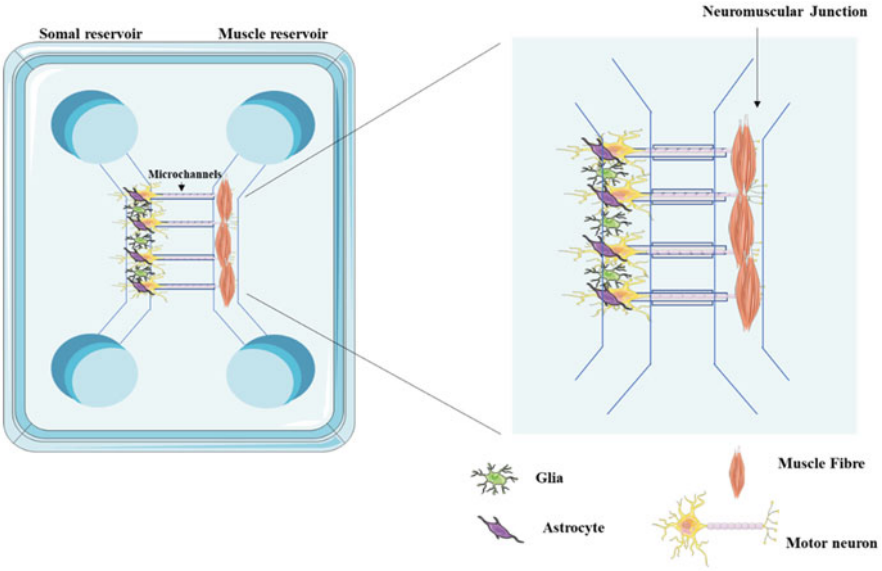

Fig. 21.4 Representative image of axonal outgrowth and allied neural cells innervating muscle fibers forming neuromuscular junction on the microfluidic device. The connecting microchannels on the device act as a platform for recapitulating motor neuron pathologies

of organelles and acidic components through axons and similarly motor neuron organoid from human-induced pluripotent stem cells (iPSCs) to form axon fascicle by interchannel elongation of axonal outgrowth on-chip [80, 81].

This microfluidic tissue compartmentalization can serve as a platform for screening of drugs (Table 21.1) and understanding the aetiogenesis of potential diseases of axonal transport dysfunction and neurodegeneration. A group of reseachers demonstrated a novel two chambered microfluidics based in vitro model to mimic the unique anatomical and cellular interactions of motor neuron–neuromuscular junction circuit. The Proximal and distal chambers were seeded with glial cells from the spinal cord and rat-derived motor neurons, which were able to manipulate the motor neuronal environment on chambers. The on-chip technique will be used to mimic neuromuscular junction for assessing prevalent motor neuron pathologies [82].

Microfluidic devices also demonstrated the glial cell-derived neurotrophic factor (GDNF)-induced muscle innervation, motor neuron, and axonal development upon the spinal cord and skeletal muscle co-culture on-chip (Fig. 21.4). The formation of the neuromuscular junction was confirmed by various analyses of neuromuscular junction activities [83].

The customized chips have less reproducibility and variability. The comparative power is also lower as desired, which can be overcome by using a reproducible Xona microfluidic chip for better results. For high-throughput translational ability, three-dimensional microfluidic approaches like ECM embedded cells depict augmented

contractility and maturity. 3-D ECM embedded neuronal cultures demonstrate increased neuronal differentiation and survival [84, 85]. A 3-D organoplate consisting of 384 well plates 40 tissue microfluidic chips was designed, which was divided into three distinctive lanes (top, middle, and bottom). The lanes were partitioned by pressure phaseguides to control overfilling of fluids. Highly predominant motor neuron axonal outgrowth was observed from the top lane of the device to further lanes. Gradient degeneration of axonal density was observed after the addition of the anticancer drug vincristine. The microfluidic device may act as a platform screening of innumerable compounds of axonal degeneration diseases like Amyotrophic lateral sclerosis [86].

21.3.2.2 ALS on Chip

Amyotrophic lateral sclerosis (ALS) is a lethal and detrimental progressive neurodegenerative disorder of idiopathic origin. It is identified by the loss of motor neurons of the cortex along with the spinal cord that results in accelerating the wasting of muscles. Manifestation of the disease is affected by dysfunctioning of voluntary activities that are characterized by muscle weakness, slurred speech followed by late-stage muscular atrophy, and respiratory failure [87, 88]. ALS being a complex multifaceted disease is important to understand the mechanistic pathways and disease pathogenesis to curb the menace of the disease. ALS primarily affects the transportation of several lipids and proteins to the neuromuscular junction [89]. Denervation of NMJ and impairment of axonal transport in ALS are characterized by fluorescently labeling of mitochondria, which get accumulated in motor nerve terminals [90].

Reconstitution of ALS disease-associated in vivo model is highly challenging, non-reproducible, and cumbersome, whereas the in vitro culture of NMJ on cell plate is relatively an effective technique that still possesses undesirable cross-contamination of non-axonal cells with axons. Microfluidic compartmentalization mimics definite axonal outgrowth and its constituents through microgrooves from one to another compartment [91]. The spatial arrangement of microfluidic devices provides the study of retrograde axonal transport and specific isolation of pure axonal RNA to understand the disease pathogenesis in a better way [92, 93]. Various other motor neuron diseases like spinal muscular atrophy (SMA) have been recapitulated in microfluidic devices, which have patient-specific feature and are challenging to assess in vivo [94].

21.4 Conclusion

Deciphering the structural and functional aspects of the human brain is an exhilarating field of experimentation. The development of the human brain, which is the most vital and complex organ of the body, is a protracted phenomenon that remains poorly understood. Unraveling this gigantic and extremely complex machinery requires innovative strategy, which can mimic the physical environment of the brain as well as offer the multi-modal efficient structural and functional

components to study and reconstitute the neural network in the form of "mini-brains". The existing two-dimensional and three-dimensional cell culture models have certain limitations that call for an innovative approach in the shape of miniaturization and microfabrication. Miniaturization has become a trend in the last 50 years and came to cover not just electronics, but also other fields like mechanical, biological, and so on. It is associated with our daily lives because even the human hair diameter lies in the range of 75 μm or our Red Blood Cells are 7.5–8.7 μm in diameter. The promising interdisciplinary approach with the collaboration of researchers from various fields of neurobiology, biotechnology, and engineering can help to understand the various events of the nervous system, from neuronal development to axon pathfinding through mimicking the brain microenvironment. The inception of the lab on-chip technologies thus holds promises for mimicking the complex brain microenvironment imparting novel platform for disease modelling and drug screening, thereby being a boon to the researchers in the field of neurobiology.

References

1. Tierney AL, Nelson CA III (2009) Brain development and the role of experience in the early years. Zero To Three 30:9
2. Oppenheim RW, Milligan CE, Von Bartheld CS (2013) Programmed cell death and neurotrophic factors. Fundament Neurosci. https://doi.org/10.1016/B978-0-12-385870-2.00018-4
3. Mukherjee N, Nandi S, Ghosh S, Garg S, Ghosh S (2020) Three-dimensional microfluidic platform with neural organoids: model system for unraveling synapses. ACS Chem Neurosci 11 (2):101–102
4. Nelson CA III, Thomas KM, De Haan M (2007) Neural bases of cognitive development. In: Handbook of child psychology, vol 2. Wiley, Hoboken, NJ
5. Rämer PC, Chijioke O, Meixlsperger S, Leung CS, Münz C (2011) Mice with human immune system components as in vivo models for infections with human pathogens. Immunol Cell Biol 89:408–416
6. Antoni D, Burckel H, Josset E, Noel G (2015) Three-dimensional cell culture: a breakthrough in vivo. Int J Mol Sci 16:5517–5527
7. Mehta G, Hsiao AY, Ingram M, Luker GD, Takayama S (2012) Opportunities and challenges for use of tumor spheroids as models to test drug delivery and efficacy. J Control Release 164:192–204
8. Van Der Helm MW, Van Der Meer AD, Eijkel JC, Van Den Berg A, Segerink LI (2016) Microfluidic organ-on-Chip Technology for blood-brain barrier research. Tissue Barriers 4: E1142493
9. Fatehullah A, Tan SH, Barker N (2016) Organoids as an in vitro model of human development and disease. Nat Cell Biol 18:246–254
10. Drost J, Clevers H (2018) Organoids in cancer research. Nat Rev Cancer 18:407–418
11. Whitesides GM (2006) The origins and the future of microfluidics. Nature 442:368–373
12. Taylor AM, Rhee SW, Tu CH, Cribbs DH, Cotman CW, Jeon NL (2003) Microfluidic multicompartment device for neuroscience research. Langmuir 19:1551–1556
13. Croushore CA, Supharoek S-A, Lee CY, Jakmunee J, Sweedler JV (2012) Microfluidic device for the selective chemical stimulation of neurons and characterization of peptide release with mass spectrometry. Anal Chem 84:9446–9452

14. Uzel SG, Amadi OC, Pearl TM, Lee RT, So PT, Kamm RD (2016) Simultaneous or sequential orthogonal gradient formation in a 3D cell culture microfluidic platform. Small 12:612–622
15. Brown JA, Pensabene V, Markov DA, Allwardt V, Neely MD, Shi M, Britt CM, Hoilett OS, Yang Q, Brewer BM (2015) Recreating blood-brain barrier physiology and structure on Chip: a novel neurovascular microfluidic bioreactor. Biomicrofluidics 9:054124
16. Wang YI, Abaci HE, Shuler ML (2017) Microfluidic blood–brain barrier model provides in vivo-like barrier properties for drug permeability screening. Biotechnol Bioeng 114:184–194
17. Cho H, Seo JH, Wong KH, Terasaki Y, Park J, Bong K, Arai K, Lo EH, Irimia D (2015) Three-dimensional blood-brain barrier model for in vitro studies of neurovascular pathology. Sci Rep 5:1–9
18. Kajtez J, Nilsson F, Fiorenzano A, Parmar M, Emnéus J (2021) 3D biomaterial models of human brain disease. Neurochem Int 147:105043
19. Björklund A, Dunnett SB (2007) Dopamine neuron systems in the brain: an update. Trends Neurosci 30:194–202
20. Carlsson A, Lindqvist M, Magnusson T (1957) 3, 4-Dihydroxyphenylalanine and 5-Hydroxytryptophan as reserpine antagonists. Nature 180:1200–1200
21. Bromberg-Martin ES, Matsumoto M, Hikosaka O (2010) Dopamine in motivational control: rewarding, aversive, and alerting. Neuron 68:815–834
22. Yu Y, Shamsi MH, Krastev DL, Dryden MD, Leung Y, Wheeler AR (2016) A microfluidic method for dopamine uptake measurements in dopaminergic neurons. Lab Chip 16:543–552
23. Senel M, Dervisevic E, Alhassen S, Dervisevic M, Alachkar A, Cadarso VJ, Voelcker NH (2020) Microfluidic electrochemical sensor for cerebrospinal fluid and blood dopamine detection in a mouse model of Parkinson's disease. Anal Chem 92:12347–12355
24. Gravitz L (2021) The promise and potential of stem cells in Parkinson's disease. Nature 597:8–10
25. Titmarsh DM, Chen H, Glass NR, Cooper-White JJ (2014) Concise review: microfluidic technology platforms: poised to accelerate development and translation of stem cell-derived therapies. Stem Cells Transl Med 3:81–90
26. Van Der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, Macleod MR (2010) Can animal models of disease reliably inform human studies? PLoS Med 7:E1000245
27. Ndyabawe K, Cipriano M, Zhao W, Haidekker M, Yao K, Mao L, Kisaalita WS (2020) Brain-on-A-Chip device for modeling multiregional networks. ACS Biomater Sci Eng 7:350–359
28. World Health Organization (2021) World Health Organization, Dementia, 2 September 2021, www.who.int/news-room/fact-sheets/detail/dementia (Accessed 28 Oct 2021).
29. Osaki T, Shin Y, Sivathanu V, Campisi M, Kamm RD (2018a) *In vitro* microfluidic models for neurodegenerative disorders. Adv Healthc Mater 7:1700489
30. Machado CB, Pluchon P, Harley P, Rigby M, Sabater VG, Stevenson DC, Hynes S, Lowe A, Burrone J, Viasnoff V (2019) In vitro modeling of nerve–muscle connectivity in a compartmentalized tissue culture device. Adv Biosyst 3:1800307
31. Ionescu A, Gradus T, Altman T, Maimon R, Avraham NS, Geva M, Hayden M, Perlson E (2019) Targeting the Sigma-1 receptor via Pridopidine ameliorates central features of Als pathology in a Sod1 G93a model. Cell Death Dis 10:1–19
32. Ionescu A, Perlson E (2019) Patient-derived co-cultures for studying Als. Nat Biomed Eng 3:13–14
33. Ali MS, Hooshmand N, El-Sayed M, Labouta HI (2021) Microfluidics for development of lipid nanoparticles: paving the way for nucleic acids to the clinic. ACS Appl Biomater. https://doi.org/10.1021/acsabm.1c00732
34. Chennampally P, Sayed-Zahid A, Soundararajan P, Sharp J, Cox GA, Collins SD, Smith RL (2021) A microfluidic approach to rescue Als motor neuron degeneration using rapamycin. Sci Rep 11:1–12
35. Gu S, Lu Y, Ding Y, Li L, Zhang F, Wu Q (2013) Droplet-based microfluidics for dose–response assay of enzyme inhibitors by electrochemical method. Anal Chim Acta 796:68–74

36. Sofela S, Sahloul S, Song Y-A (2021) Biophysical analysis of drug efficacy on C. Elegans models for neurodegenerative and neuromuscular diseases. PLoS One 16:E0246496
37. De Oliveira TR, Erbereli CR, Manzine PR, Magalhães TN, Balthazar ML, Cominetti MR, Faria RC (2020) Early diagnosis of Alzheimer's disease in blood using a disposable electrochemical microfluidic platform. ACS Sens 5:1010–1019
38. Park J, Lee BK, Jeong GS, Hyun JK, Lee CJ, Lee S-H (2015) Three-dimensional brain-on-A-Chip with an interstitial level of flow and its application as an in vitro model of Alzheimer's disease. Lab Chip 15:141–150
39. Fantuzzo JA, De Filippis L, Mcgowan H, Yang N, Ng Y-H, Halikere A, Liu J-J, Hart RP, Wernig M, Zahn JD (2017) M neurocircuitry: establishing in vitro models of Neurocircuits with human neurons. Technology 5:87–97
40. Maoz BM, Herland A, Fitzgerald EA, Grevesse T, Vidoudez C, Pacheco AR, Sheehy SP, Park T-E, Dauth S, Mannix R (2018) A linked organ-on-Chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. Nat Biotechnol 36:865–874
41. Tourovskaia A, Li N, Folch A (2008) Localized acetylcholine receptor clustering dynamics in response to microfluidic focal stimulation with Agrin. Biophys J 95:3009–3016
42. Taylor AM, Jeon NL (2010) Micro-scale and microfluidic devices for neurobiology. Curr Opin Neurobiol 20:640–647
43. Hosmane S, Yang IH, Ruffin A, Thakor N, Venkatesan A (2010) Circular compartmentalized microfluidic platform: study of axon–glia interactions. Lab Chip 10:741–747
44. Taylor AM, Blurton-Jones M, Rhee SW, Cribbs DH, Cotman CW, Jeon NL (2005) A microfluidic culture platform for CNS axonal injury, regeneration and transport. Nat Methods 2:599–605
45. Majumdar D, Gao Y, Li D, Webb DJ (2011) Co-culture of neurons and glia in a novel microfluidic platform. J Neurosci Methods 196:38–44
46. Park J, Koito H, Li J, Han A (2009) Microfluidic compartmentalized co-culture platform for CNS axon myelination research. Biomed Microdevices 11:1145–1153
47. Shi M, Majumdar D, Gao Y, Brewer BM, Goodwin CR, Mclean JA, Li D, Webb DJ (2013) Glia co-culture with neurons in microfluidic platforms promotes the formation and stabilization of synaptic contacts. Lab Chip 13:3008–3021
48. Adriani G, Ma D, Pavesi A, Goh EL, Kamm RD (2015) Modeling the blood-brain barrier in a 3D triple co-culture microfluidic system. In: 2015 37th annual international conference of the IEEE engineering in medicine and biology society (EMBC). IEEE, New York, pp 338–341
49. Yong Y, Hughes C, Deppmann C (2020) A microfluidic culture platform to assess axon degeneration. Axon degeneration. Springer, New York
50. Bolognin S, Fossépré M, Qing X, Jarazo J, Ščančar J, Moreno EL, Nickels SL, Wasner K, Ouzren N, Walter J (2019) 3D cultures of Parkinson's disease-specific dopaminergic neurons for high content phenotyping and drug testing. Adv Sci 6:1800927
51. Liu J, Sternberg AR, Ghiasvand S, Berdichevsky Y (2018) Epilepsy-on-A-Chip system for antiepileptic drug discovery. IEEE Trans Biomed Eng 66:1231–1241
52. Osaki T, Uzel SG, Kamm RD (2018b) Microphysiological 3D model of amyotrophic lateral sclerosis (Als) from human Ips-derived muscle cells and optogenetic motor neurons. Sci Adv 4: Eaat5847
53. Ruiz A, Joshi P, Mastrangelo R, Francolini M, Verderio C, Matteoli M (2014) Testing Aβ toxicity on primary CNS cultures using drug-screening microfluidic chips. Lab Chip 14:2860–2866
54. Shin Y, Choi SH, Kim E, Bylykbashi E, Kim JA, Chung S, Kim DY, Kamm RD, Tanzi RE (2019) Blood–brain barrier dysfunction in a 3D in vitro model of Alzheimer's disease. Adv Sci 6:1900962
55. Wevers NR, Van Vught R, Wilschut KJ, Nicolas A, Chiang C, Lanz HL, Trietsch SJ, Joore J, Vulto P (2016) High-throughput compound evaluation on 3D networks of neurons and glia in a microfluidic platform. Sci Rep 6:1–10

56. Yi H-G, Jeong YH, Kim Y, Choi Y-J, Moon HE, Park SH, Kang KS, Bae M, Jang J, Youn H (2019) A bioprinted human-glioblastoma-on-A-Chip for the identification of patient-specific responses to chemoradiotherapy. Nat Biomed Eng 3:509–519
57. Takeuchi A, Nakafutami S, Tani H, Mori M, Takayama Y, Moriguchi H, Kotani K, Miwa K, Lee J-K, Noshiro M (2011) Device for co-culture of sympathetic neurons and cardiomyocytes using microfabrication. Lab Chip 11:2268–2275
58. Neto E, Alves CJ, Sousa DM, Alencastre IS, Lourenço AH, Leitão L, Ryu HR, Jeon NL, Fernandes R, Aguiar P (2014) Sensory neurons and osteoblasts: close partners in a microfluidic platform. Integr Biol 6:586–595
59. Lei Y, Li J, Wang N, Yang X, Hamada Y, Li Q, Zheng W, Jiang X (2016) An on-Chip model for investigating the interaction between neurons and cancer cells. Integr Biol 8:359–367
60. Crawford LK, Caterina MJ (2020) Functional anatomy of the sensory nervous system: updates from the neuroscience bench. Toxicol Pathol 48:174–189
61. Koop LK, Tadi P (2021) Neuroanatomy, sensory nerves. In: StatPearls [Internet]. StatPearls Publishing, Treasure Island, FL. [Accessed 31 Jul 2021]. Available from https://www.ncbi.nlm.nih.gov/books/NBK539846/
62. Newbern JM (2015) Molecular control of the neural crest and peripheral nervous system development. Curr Top Dev Biol 111:201–231
63. Hanani M (2005) Satellite glial cells in sensory ganglia: from form to function. Brain Res Rev 48:457–476
64. Arvidson B (1979) Distribution of intravenously injected protein tracers in peripheral ganglia of adult mice. Exp Neurol 63:388–410
65. Davis MP (2012) Drug management of visceral pain: concepts from basic research. Pain Res Treat 2012:265605
66. Umans BD, Liberles SD (2018) Neural sensing of organ volume. Trends Neurosci 41:911–924
67. Kim HN, Choi N (2019) Consideration of the mechanical properties of hydrogels for brain tissue engineering and brain-on-A-Chip. Biochip J 13:8–19
68. Chaudhuri O (2017) Viscoelastic hydrogels for 3D cell culture. Biomater Sci 5:1480–1490
69. Johnson BN, Lancaster KZ, Hogue IB, Meng F, Kong YL, Enquist LW, Mcalpine MC (2016) 3D printed nervous system on a Chip. Lab Chip 16:1393–1400
70. Sakai K, Shimba K, Kotani K, Jimbo Y (2017) A co-culture microtunnel technique demonstrating a significant contribution of unmyelinated Schwann cells to the acceleration of axonal conduction in Schwann cell-regulated peripheral nerve development. Integr Biol 9:678–686
71. Anderson WA, Willenberg AR, Bosak AJ, Willenberg BJ, Lambert S (2018) Use of a capillary alginate gel (Capgel™) to study the three-dimensional development of sensory nerves reveals the formation of a rudimentary Perineurium. J Neurosci Methods 305:46–53
72. Sharma AD, Mccoy L, Jacobs E, Willey H, Behn JQ, Nguyen H, Bolon B, Curley JL, Moore MJ (2019) Engineering a 3D functional human peripheral nerve in vitro using the nerve-on-A-Chip platform. Sci Rep 9:1–12
73. Tsantoulas C, Farmer C, Machado P, Baba K, Mcmahon SB, Raouf R (2013) Probing functional properties of nociceptive axons using a microfluidic culture system. PLoS One 8: E80722
74. Vysokov N, Mcmahon SB, Raouf R (2019) The role of Na V channels in synaptic transmission after Axotomy in a microfluidic culture platform. Sci Rep 9:1–13
75. Stifani N (2014) Motor neurons and the generation of spinal motor neurons diversity. Front Cell Neurosci 8:293
76. Eccles J, Eccles RM, Lundberg A (1957) The convergence of monosynaptic excitatory afferents on to many different species of alpha Motoneurones. J Physiol 137:22–50
77. Eccles JC, Eccles RM, Iggo A, Lundberg A (1960) Electrophysiological studies on gamma motoneurones. Acta Physiol Scand 50:32–40

78. Jaiswal MK (2014) Selective vulnerability of Motoneuron and perturbed mitochondrial calcium homeostasis in amyotrophic lateral sclerosis: implications for Motoneurons specific calcium dysregulation. Mol Cell Ther 2:1–15
79. Campenot RB (1977) Local control of neurite development by nerve growth factor. Proc Natl Acad Sci 74:4516–4519
80. Altman T, Maimon R, Ionescu A, Pery TG, Perlson E (2020) Axonal transport of organelles in motor neuron cultures using microfluidic chambers system. J Vis Exp E60993
81. Osaki T, Chow SYA, Nakanishi Y, Hernandez J, Kawada J, Fujii T, Ikeuchi Y (2020) Three-dimensional motor nerve organoid generation. J Vis Exp E61544
82. Southam KA, King AE, Blizzard CA, Mccormack GH, Dickson TC (2013) Microfluidic primary culture model of the lower motor neuron–neuromuscular junction circuit. J Neurosci Methods 218:164–169
83. Zahavi EE, Ionescu A, Gluska S, Gradus T, Ben-Yaakov K, Perlson E (2015) A compartmentalized microfluidic neuromuscular co-culture system reveals spatial aspects of Gdnf functions. J Cell Sci 128:1241–1252
84. Brännvall K, Bergman K, Wallenquist U, Svahn S, Bowden T, Hilborn J, Forsberg-Nilsson K (2007) Enhanced neuronal differentiation in a three-dimensional collagen-Hyaluronan matrix. J Neurosci Res 85:2138–2146
85. Shima A, Morimoto Y, Sweeney HL, Takeuchi S (2018) Three-dimensional contractile muscle tissue consisting of human skeletal myocyte cell line. Exp Cell Res 370:168–173
86. Spijkers XM, Pasteuning-Vuhman S, Dorleijn JC, Vulto P, Wevers NR, Pasterkamp RJ (2021) A directional 3D neurite outgrowth model for studying motor axon biology and disease. Sci Rep 11:1–15
87. Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, Burrell JR, Zoing MC (2011) Amyotrophic lateral sclerosis. Lancet 377:942–955
88. Masrori P, Van Damme P (2020) Amyotrophic lateral sclerosis: a clinical review. Eur J Neurol 27:1918–1929
89. Millecamps S, Julien J-P (2013) Axonal transport deficits and neurodegenerative diseases. Nat Rev Neurosci 14:161–176
90. De Vos KJ, Hafezparast M (2017) Neurobiology of axonal transport defects in motor neuron diseases: opportunities for translational research? Neurobiol Dis 105:283–299
91. Lu X, Kim-Han JS, O'Malley KL, Sakiyama-Elbert SE (2012) A microdevice platform for visualizing mitochondrial transport in aligned dopaminergic axons. J Neurosci Methods 209:35–39
92. Briese M, Saal L, Appenzeller S, Moradi M, Baluapuri A, Sendtner M (2016) Whole transcriptome profiling reveals the Rna content of motor axons. Nucleic Acids Res 44:E33–E33
93. Sahoo PK, Smith DS, Perrone-Bizzozero N, Twiss JL (2018) Axonal Mrna transport and translation at a glance. J Cell Sci 131:Jcs196808
94. Santoso JW, Mccain ML (2020) Neuromuscular disease modeling on a Chip. Dis Model Mech 13:Dmm044867

Skin-on-Chip

22

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Abstract

In the last few decades, skin bioengineering has undergone several advancements. The need, understanding, and importance of developing artificial skin, i.e. in vitro human skin models (HSMs) such as static and dynamic perfusion-based skin-on-chip (SOC) models for studying the effect of cosmetics, skin diseases, and drug discovery, have started budding in recent years. SOCs are shown to be more realistic, high throughput, and less expensive alternatives to animal models for pre-clinical testing. Thus, fabrication, development, characterization, and validation of various in vitro HSMs like SOC came into the subject of skin tissue engineering, regenerative medicine, and diagnostics. The ideology of SOC proves the basic principle of 3Rs, i.e. replace, reduce, and refine (RRR). It satisfies the morphological, biomechanical, and functional resemblance to human skin. It has been validated by applications of cosmetics and pharmaceutical products, significantly demonstrating its efficacy, delivery, and toxicity. In this book chapter, all the mentioned parameters are discussed sequentially. An outlook on SOC impact, regulations, IPR followed by challenges, and current and future perspectives is also presented in the later section of this chapter.

Keywords

SOC · Pre-clinical testing · Microfluidics · Fabrication · Validation · Regulations · Skin diseases

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22.1 Introduction, Need, and Importance

The skin is a multi-layered and complex organ of the human body. It conducts numerous physiological functions, such as fluid homeostasis, thermoregulation, immune defence, vitamin D synthesis, and sensory detection. It forms an efficient barrier against environmental pathogens, UV radiations, and toxic chemicals [1]. Skin, which is the outermost barrier, is affected through the presence of air pollutants that consist of particulate matter, volatile compounds, and aromatic hydrocarbons. Skin is harmed via the oxidative stress that is induced through these air pollutants. Atopic dermatitis, eczema, and pigmentation affect aesthetic appeal of the skin [2]. Exposure of skin to ultraviolet radiation has been associated with extrinsic ageing and squamous cell carcinoma [3]. Smoke contributes to premature ageing. Additionally, same has been reported to increase the incidence of psoriasis and acne.

Globally, around 1.9 billion people are affected by skin diseases and subcutaneous disorders [4]. Analysing the global burden, acne vulgaris affects 40–80% of young population. Elder population in the age range of 30–50 years largely experiences dermatitis, while keratinocyte carcinoma and melanoma are more prominent in population with age greater than 80 years [5]. Additionally, protection from UV radiations is sought by the people of all ages. Additionally, the skin, being the largest organ of our body, offers a promising pathway for treating skin and other health problems via topical drug delivery through transdermal systems [6]. This has led to an increase in the demand for cosmetic as well as other skin-based pharmaceutical products. An extensive time is invested by researchers on developing drugs for treating skin problems. However, it is challenging and expensive to develop transdermal drug delivery systems as skin is efficient in obviating unwanted invaders. Moreover, the developed drugs and cosmetics have to be tested and validated to meet regulatory requirements and thus to be declared safe for the end user.

Skin equivalent and SOC models that enable a detailed understanding of healthy and diseased skins may serve as an ideal pre-clinical tool to evaluate cosmetic and medicated skin products for skin adornment, skin diseases, wounds, tumours, allergies, etc. and to study the effect of environmental stimuli. Such models can also be useful as models for basic research. They can thus act as effective alternatives to animal investigations [7, 8]. Various cell types, such as primary cells and induced pluripotent stem cells, are used to construct skin models. Two-dimensional (2D) models involving keratinocytes co-cultured with immune cells and dermal fibroblasts have been developed. However, they do not mimic the complicated skin structure as the skin comprises of hair follicles, melanocytes, blood vessels, and multi-layered structure. Furthermore, the 2D models cannot replicate cell-cell and cell-matrix interactions [9]. 2D cell-based assays provide information about advantages and ill effects of potential drugs in humans, but cannot predict the metabolic behaviour of these drugs [10, 11]. Therefore, 3D skin equivalents are being constructed to replicate the three distinct layers of skin, viz., epidermis,

dermis, and subcutaneous adipose tissue, along with the tight and gap junctions that maintain the integrity and functions of skin tissue.

In this chapter, first we will discuss the concept, need for, and importance of in vitro skin models (Sect. 22.1). In Sect. 22.2, we have discussed basic structure and function of human skin, cellular microenvironment followed by evolution, types, variation, and minimal requirements for SOC modelling. Further, in Sect. 22.3, the components and development of the SOC model are explained in a comprehensive manner. This includes understanding of skin bioengineering-related biomaterials, material of construction (MOC), skin cells and their types, reagents required for SOC development, and fabrication of microfluidic devices, which are also well encapsulated in the Fig. 22.3 and Table 22.1. It further details down characterization of developed skin equivalent by use of SOC devices and their validation parameters and studies required to evaluate it as a pre-clinical testing and alternative to animal models (Table 22.2). Later in this part, healthy and disease-specific skin biomarkers which serve as the end point of SOC models are listed in the Tables 22.3 and 22.4. Further in Sect. 22.4, understanding the advantages and implementations has been elaborated, followed by Sect. 22.5, mentioning impact of SOC technology, along with related regulations and IPR issues. Later in Sect. 22.6, it consists largely of challenges associated with the SOC technology. The last Sect. 22.7 ends with discussions on current and future aspects of microfluidic SOC technology.

22.1.1 An Alternative to Animal Testing

Animal models are the backbone of biomedical research due to their ability to partially mimic the human systems. Animals such as rats, mice, fishes, rabbits, birds (mainly chicken), guinea pigs, frogs, primates, dogs, etc. have been primarily used for assessing the efficacy and toxicity of biomedical products. However, the use of animals as pre-clinical models has been always met with contradictory preferences, with many questioning the suitability and ethics of animal usage. Countries around the world have adapted the concept of 3Rs, which refers to replacement, reduction, and refinement of animal usage in experimentation. Still, the use of animals has not been significantly reduced and more than 100 million animals are annually used for various testing [12]. The high similarity of genetic material and the presence of whole organ systems render animals as an ideal choice for predicting the effect of target molecules in humans. Thus, absolute replacement of animals for biomedical testing is difficult.

However, the problems associated with the usage of animals have been mitigated through the development of in silico models, in vitro cell models, simulation of biological processes, and creating virtual organ maps, along with the involvement of human volunteers. An alternative way is to use lower invertebrate organisms as models for studying specific aspects, such as using *E. coli*, *Neurospora* sp., and *Saccharomyces* sp. as models for genetic and molecular biology studies, hydra and cnidaria for regeneration studies, *Drosophila melanogaster* for genetic and developmental studies, etc. In case of studies involving complex understanding, evaluation,

Table 22.1 Detailed summary table showing microfabrication and development of different microfluidic skin-on-a-chip (SOC) models

S. No.	In vitro SOC models	Fabrication	Duration	3D- culture type	Skin layer	Skin cell type used	Biomaterial/ Scaffold	Support membrane (filter)	Membrane Pore size (μm), pore density	Device material	Flow	Reference
1	In situ (full-thickness bilayer tissue model)	Cut and assemble manufacturing	4	Co-culture	Epidermal, dermal	Primary human fibroblasts and immortalized keratinocytes (HaCaT)	Fibrin	PC	5 μm	PDMS	Perfusion	[137]
2	In situ (full-thickness bilayer tissue model)	Micro-lithography	7	Co-culture	Epidermal, dermal	Primary fibroblasts and keratinocytes	Collagen	Not stated	0.4 μm	PDMS	Pumpless, gravity-driven	[138]
3	In situ (full-thickness bilayer tissue model)	Soft lithography	7	Co-culture	Epidermal, dermal	Primary fibroblasts and keratinocytes	Collagen	Polyester	Not stated	PDMS	Pumpless, gravity-driven	[139]
4	In situ (monolayer tissue model)	Soft lithography	15	monoculture	Epidermal	Immortalized HaCaT keratinocytes	None	PET	1 μm	PDMS	Perfusion	[140]
5	In situ (full-thickness bilayer tissue model)	CNC micro-milling	4	Co-culture	Epidermal, dermal	Primary human fibroblasts and immortalized N/TERT keratinocytes	Fibrin + PEG	PC	1 μm	PMMA	Perfusion	[21]
6	In situ (full-thickness bilayer tissue model)	Soft lithography	7	Co-culture	Epidermal, dermal	Primary fibroblasts and keratinocytes	Collagen	Not stated	Not stated	PDMS and glass	Perfusion	[141]
7	In situ (full-thickness bilayer tissue model)	Soft lithography	5–7	Co-culture	Epidermal, dermal	Primary fibroblasts and keratinocytes	Collagen	Not stated	Not stated	PDMS	Pumpless, gravity-driven	[142]

8	In situ (full-thickness bilayer tissue model)	micro-lithography	5–7	Co-culture	Epidermal, dermal	Primary fibroblasts and keratinocytes	Collagen (different sources)	Not stated	Not stated	PDMS	Pumpless, gravity-driven	[143]
9	In situ	Soft lithography	10	Co-culture	Epidermal, dermal	Primary fibroblasts and keratinocytes + HUVECs	Collagen	PC	Not stated	PDMS	Pumpless, gravity-driven	[52]
10	In situ	micro-lithography	17	Co-culture	Epidermis with immune cells	Immortalized HaCaT keratinocytes (KCs) and U937 for dendritic cells	None	PET	0.4 μm, 4×10^6 pore/cm^2	PMMA, PS, PDMS	Perfusion (negative pressure)	[27]
11	In situ (full-thickness 3-layered model)	Conventional soft lithography	3	Co-culture	Epidermal, dermal	Immortalized HS27 fibroblasts and HaCaT keratinocytes and HUVECs	None	PET (X2)	0.4 μm	PDMS	Pumpless, gravity-driven	[94]
12	Transferred (full-thickness model)	Soft lithography	1	Skin explant	Epidermal, dermal, hypodermis	Blood cells + human biopsy	Biopsy	Red blood cell filter	–	PDMS	Static	[133]
13	Transferred	Soft lithography	1	Co-culture	Epidermal, dermal	Primary fibroblasts and keratinocytes	Collagen	PC	5 μm	PDMS	Pumpless, gravity-driven	[93]
14	Transferred	Transwell inserts	1–2	Co-culture	Epidermal, dermal	L929 murine fibroblasts and EpiDerm™	None	Not stated	3 μm	Commercial platform + transwell insert	Perfusion	[144]
15	Transferred	Transwell inserts and micropump	14	Co-culture	Epidermal, dermal, hair follicle	EpiDermFT™ (commercial) + ex-vivo subcutaneous tissue	EpiDermFT™ (commercial)	Not stated	Not stated	PDMS	Perfusion, on-chip micropump	[99]

(continued)

Table 22.1 (continued)

S. No.	In vitro SOC models	Fabrication	Duration	3D- culture type	Skin layer	Skin cell type used	Biomaterial/ Scaffold	Support membrane (filter)	Membrane Pore size (μm), pore density	Device material	Flow	Reference
16	Transferred	Micro-lithography	28	Co-culture	4 organs including skin (Epidermal, dermal)	Human Biopsy	Biopsy	Not stated	0.4 μm	PDMS	Perfusion, on-chip micropump	[145]
17	Transferred	Photolithography	14	Co-culture	Liver microtissues, skin tissue	Human Biopsy	Biopsy	Not stated	0.4 μm	PDMS	Perfusion, on-chip micropump	[146]

Table 22.2 Detailed summary table showing characterization, validation, and applications of different microfluidic skin-on-a-chip (SOC) models

S. No.	In vitro Skin-on-a-chip model type	Characterization studies	Validation studies	Applications	Reference
1	In situ (full-thickness bilayer tissue model)	Cell viability, cell distribution and arrangement, rheological studies, mathematical proof for parallel flow model	Gel height estimation, study of injectability, parallel flow and viscosity vs. shear rate values estimation, fluorescence microscopy image acquisition	Industrial relevant model (mathematical parallel flow compartmentalized model for generating complex 3D structural and physiological similar human skin)	[137]
2	In situ (full-thickness bilayer tissue model)	H&E staining, immunostaining, quantitative analysis of real-time quantitative PCR	Test of sorafenib drug using this device by gene expression of the key proteins in model	Pre-clinical drug and toxicity testing model	[138]
3	In situ (full-thickness bilayer tissue model)	H&E staining (observing thickness of skin equivalent), quantitative analysis (qRT-PCR) of expression of healthy skin biomarkers like filaggrin, involucrin, Keratin 10, and Laminin alpha-5	Evaluation of curcuma longa leaf extract (CLLE) efficacy using developed model	Cosmetics formulations testing model	[139]
4	In situ (monolayer tissue model)	Cell viability and permeation assays	Parallel permeation assays with fluorescent-dye-labelled dextran as a tracer and potassium dichromateas an irritant.	Photolithography-free, biosensing, drug development model	[140]
5	In situ (full-thickness bilayer tissue model)	Immunostaining & image analysis, two-Photon microscopy, confocal Raman spectroscopy and analysis, TEER and permeation assays & analysis	Dynamic perfusion,control of the microenvironment, enhanced epidermal morphogenesis, differentiation, barrier function	Scalable system, higher throughput and automation of culture, testing protocols, low-cost alternatives to animal and clinical studies (drug screening, toxicology)	[21]

(continued)

Table 22.2 (continued)

S. No.	In vitro Skin-on-a-chip model type	Characterization studies	Validation studies	Applications	Reference
6	In situ (full-thickness bilayer tissue model)	Uniaxial cyclic stretching to induce wrinkles(ageing)	Staining methods (H&E, IHC, Masson Trichrome, Sirius Red/Fast Green)	Wrinkled skin-on-a-chip model to test anti-wrinkle ingredients and cosmetics efficacy	[141]
7	In situ (full-thickness bilayer tissue model)	Not specified	Staining methods (H&E, IHC, Masson Trichrome, Sirius Red/Fast Green)	On-chip culture and differentiation of 3D skin equivalent	[142]
8	In situ (full-thickness bilayer tissue model)	SEM for collagen matrices and contraction (21 days)	Staining methods (H&E, IHC, Masson Trichrome, Sirius Red/Fast Green)	SOC model using different collagen sources	[143]
9	In situ	Design and operating conditions of skin chip, cell viability assay, estimation of parameters for mass transfer model, histological and immunohistochemical analysis	Cell growth and differentiation studies, transport/permeability studies	Interaction between different components of the skin tissue, physiologically realistic platform for testing skin reaction to cosmetic products and drugs	[52]
10	In situ	Cell viability, TEER measurement, paracellular permeability, Immunofluorescence (IF) staining of intercellular tight junctions	Cell stimulation and secreted cytokine detection, Effect of UV irradiation on TEER, LPS-induced expression of IL-6and IL-1β	3-tiered culture differential stimulation for investigating the role of KC layer as a protection barrier to chemical/biological hazards, skin sensitization, and toxicity studies	[27]

11	In situ (full-thickness 3-layered model)	Cell labelling (orientation of 3 cell types), immunocytochemical tight junction staining, paracellular permeability (FITC)-dextran, TNF-α-Inflammation induction (IL-1β, IL-6, and IL-8 analysis by RT-PCR, multiplex assay)	Dexamethasone (Dex) treatment to TNF-α -induced inflammation (IL-1β, IL-6, and IL-8 analysis by ELISA, RT-PCR, immunocytochemical tight junction staining)	Skin inflammation and oedema model for testing the toxicity of cosmetics or drugs.	[94]
12	Transferred (full thickness)	*S. aureus* infections followed by neutrophil migration and its counting and integration of green, fluorescent intensity processed using an ImageJ	Effect of penicillin treatment on *S. aureus* inoculated MSTCs	Model used for distinguishing between cellulitis and pseudo-cellulitis patient samples, SSTI diagnosis, monitoring, and treatment optimization	[133]
13	Transferred	H&E and immunofluorescence (IF) staining	Doxorubicin treatment and analyses of transdermal transport and barrier integrity by fluorescence spectrometer and H&E staining	Drug toxicity testing	[20]
14	Transferred	Biochip encapsulation design, dual fluidic configurations, capability of the intelligent mobile lab for in vitro diagnostics (IMOLA-IVD) to be used on complex 3D tissue models	Reaction to Sodium Dodecyl Sulphate Medium, TEER and Extracellular acidification rate (EAR) measurements	Tool for creating non-invasive automated cellular assays, skin toxicity studies, maintaining stable cultures on the chip, monitoring metabolic parameters, and revealing tissue breakdown over time by automated TEER measurement system that also preserves an ALI	[144]
15	Transferred	Dynamically perfused chip-based bioreactor platform capable of applying variable mechanical shear stress and extending culture periods	H and E immunostaining	Toxicity testing and compound screening	[99]

(continued)

Table 22.2 (continued)

S. No.	In vitro Skin-on-a-chip model type	Characterization studies	Validation studies	Applications	Reference
16	Transferred	Characterization of fluid dynamics, cytotoxicity and metabolic activity, real-time qPCR, immunostaining	TEER measurement, 3D imaging two-photon microscopy, Immunofluorescence staining of RPTEC/TERT-1 cell monolayers	In vitro absorption, distribution, metabolism and excretion (ADME) and repeated dose toxicity testing model	[145]
17	Transferred	Cell viability and protein expression were assessed by immunohistochemistry (IHC) and quantitative PCR (qPCR)	Troglitazone for repeated dose-dependent testing and validation by IHC and real-time qPCR endpoint analyses	Drug testing model	[146]

Table 22.3 Healthy skin biomarkers specific to epidermis and dermis regions

Human skin regions	Biomarker	Function	Reference
Epidermis			
Basement membrane	Ki67	Proliferation marker	[174]
	Hemidesmosomes	Adhesion markers	[175]
Suprabasal keratinocytes	Involucrin	Early differentiation	[176]
	Keratins 1 and 10,		
Granular keratinocytes	Profilaggrin	Terminal differentiation	
	Keratohyalin granules		
Stratum corneum	Transglutaminase, Cytokeratin 10	Cornification	
SC Lipid barrier	Phospholipids, Cholesterol sulphate, Glycosphingolipids, Ceramides, Free fatty acids, Cholesterol, Triglycerides, and Cholesterol Esters	Lipid barrier	[177]
Dermal-Epidermal Junction	Laminin 5, Collagen-IV, Collagen VII, Fibrillin-1		[178, 179]
Dermis	Fibrillin 1, Pro-collagen I	Dermal ECM	[178]
	Decorin	Dermal ECM	[180]
	Collagen VII A1 (COL 7 A1) and Collagen IV A1(COL 4 A1)	Fibroblast (Fbs) markers	[181]

and discovery of drugs, these organisms are often not an ideal choice for predicting the actual outcomes in humans or higher vertebrates.

In the current pharmaceutical infrastructure, development of new drugs costs about 2.5 billion USD, whereas the process spans over a duration of 13.5 years. However, despite the heavy investment of time and cost, the failure rate of drugs in clinical trials is above 90% [13]. Technological advancements in recent years have enabled partial replacement of animal trials with three-dimensional (3D) skin models. 3D skin models, such as human skin equivalents or skin-on-a-chips (SOCs) etc., can mimic the physiology of actual skin and more accurately extrapolate the in vivo microenvironment. Such models are rapidly evolving. The SOCs are being employed for risk assessment of drugs, industrial chemicals [14], and cosmetic ingredients [15]. SOCs recapitulate the 3D cell microenvironment, lipid composition, cellular organization, and polarity much better than the traditional 2D monolayer cell culture systems [16, 17]. Integration of microfluidics with SOC devices has improved the quality and functionality of these chips by a high magnitude. The chief advantages include improved mimicking of fluid conditions within the organs and improved delivery of nutrients and drugs, which may help in studying cell morphology, differentiation, cell migration, cell-cell interaction, etc., which are absent in static cultures [18, 19]. The SOCs developed over the past decade have exhibited

Table 22.4 Biomarkers specific to in vitro human diseased skin models

Human skin diseases	Type of model and skin cells	Biomarkers	Regulation with disease	Description and mechanism	Method of detection	Inducers	Reference
Psoriasis	Organotypic co-culture on a collagen matrix with normal human keratinocytes	Procaspase and Caspase-14	Absent or reduced markers	Loss of keratinocyte terminal differentiation	Immunoblot, IHC	Vitamin D3	[182]
		Keratin 1 and 10, Loricrin, and Trans-glutaminase 1					
		Ki-67	Upregulated marker	Increase in proliferation			
	Reconstructed Human epidermis with normal human keratinocytes	Occludin	Discontinuous expression with enlarged intracellular spaces	Decrease of cellular adhesion in differentiated keratinocytes	Immuno-fluorescence (IF)	IL-17, TNF-α	[183]
		C/EBPβ, LCN-2 and HBD2					[183]
	3D skin equivalent with T cells with Th1 and Th17 polarized CD4+ cells	DEFB4, PI3, LCE3A, KRT16 and S100A7	Upregulated	Psoriasis-associated genes	qPCR, Immunostaining	anti-CD3/CD28 mAb-coated beads	[90]
		Filaggrin, Involucrin	Downregulated	Differentiation markers			
		IL23, IL6, IL8, CXCL	Upregulated	Chemokines and pro-inflammatory cytokines increase upon direct and indirect contact from T cells			
	HaCaT cell culture	IL28RA	Absent in lesioned tissue	Being a proliferation inhibitor, it disappears in lesioned tissue where excessive proliferation is observed	Immuno-histochemistry (IHC) and western blotting	IL-29	[184]
Oedema	SOC model with HaCaT cells, Fbs, HUVECs	IL-1β, IL-6, and IL-8	Upregulated	Inflammatory cytokine	ELISA	TNF-α	[26]
		Zonula occludens (ZO)	Downregulated	Loss of tight junction protein causes fluid accumulation and swelling	IHC (tight junction staining)		

Allergic contact dermatitis	3D co-culture with HaCaT and U937 dendritic cell lines	IL-6 and IL-1β	Upregulated	Dendritic cell activation	Sandwich immunoassay	LPS and Nickel Sulphate	[27]
	EpiCS®, SkinEthic™, EpiDerm™, UVMC-EE	IL-18	Dose-dependent upregulation	IL-18 is an inducer of IFN-γ during immune response, thus used to rank potency of sensitizers	ELISA	Contact sensitizers	[185]
	EpiDerm™ with Neonatal foreskin NHEKs	ATF3, IL-8	Not reduced	NrF2 knockdown has no effect	EpiSensA assay: Can identify lipophilic irritants and pre/pro haptens	Contact sensitizers, NrF-2 siRNA	[186]
		DNAJB4 and GCLM	Downregulated	NrF2 knockdown affects expression			
	RHE (Episkin® dermal support with integrated Langerhan cells) with mammary skin NHEKs, CD 34+ derived dendritic cells	IL-1β mRNA	Upregulated for certain sensitizers	Langerhan cell activation	Langerin IHC	TNF-α, IL-1β, and other contact irritants, UV irradiation	[187]
					RT PCR		
Atopic dermatitis	Reconstructed Human epidermis	Barrier function	Decreased	Barrier function decreased by cholesterol depletion	TEER with lucifer yellow dye	Methyl-β-cyclodextrin	[188]
		FLR, LOR	Downregulated markers	Compromised keratinocyte differentiation	qRT-PCR, IHC	IL-4, IL-13, and IL-25	
		CA2, NELL2	Upregulated markers	Atopic dermatitis-associated genes			
	Leiden epidermal model (LEM)	K10, LOR	Decreased expression	Compromised keratinocyte differentiation	IHC, Western blot, RT-qPCR	IL-4, IL-13, or IL-31, TNF-α	[189]
		Ki-67	Upregulated	Increased basal proliferation except with IL-13			
		CerS	Upregulated	Sc lipid profile modification, increase coupled with cholesterol depletion	LC-MS Cer profiling		

(continued)

Table 22.4 (continued)

Human skin diseases	Type of model and skin cells	Biomarkers	Regulation with disease	Description and mechanism	Method of detection	Inducers	Reference
Vitiligo	RHPE with melanocytes	Melan-A	Downregulated	Melanocyte detachment from basement membrane	Immuno-fluorescence (IF)	IFN-γ and TNF-α	[190]
		E-cadherin	Upregulated	Increase in soluble marker levels indicates loss form DEJ			
		Caspase-3	Upregulated	Increase in apoptosis	TUNEL assay		
Fibrosis	Vascularized HSE	ACTA2 mRNA and a-SMA	Upregulated	Fibroblast to myofibroblast transition	qPCR, Immunostaining	TGFβ	[191]
		PAI1 and SMAD7		TGFb target genes			
		COL1A1, COL1A2 and fibronectin mRNA		ECM components generated by fibroblasts			
		Collagen 1 protein					
Melanoma	Organotypic skin equivalent on Alvetex® with patient-derived primary keratinocytes, melanoma cell lines (SK-mel-28, and WM35), and neonatal foreskin fibroblasts	Collagen type IV, Collagen type VII,	Initial intact expression and progressive disruption coupled with metastasis	Initial localization of melanoma cells above collagens IV and VII of the DEJ followed with clear invasion and metastasis into the dermal compartment	IHC	Melanoma cells seeded in the dermal equivalent	[192]
		Melan-A	Intact expression				
	3D organotypic model with SBCL2, WM115, 451-LU melanoma cell lines	Ki-67	seen in peripheral subpopulation of spheroid	Proliferation marker	IHC	Non-vascularized spheroids inserted in dermal scaffold	[193]
		ABCB5 and JARID1B		Chemoresistance proteins			
		DNA breaks	Central subpopulation of spheroid	Apoptotic marker	TUNEL assay		
Squamous Cell Carcinoma	3D bioprinted model with A431 cSCC keratinocytes	S100A7, S100A8, S100A9, KRT6A, SERPINB3, SERPINB4, and PI3	Upregulated markers	Modulation found to correlate with in vivo levels of said markers and are responsible for	Fluorescence (FL) microscopy using Zs-GFP and tdT-RFP	_	[194]

				over-proliferation, invasion, and metastasis			
		IL-7	Downregulated marker	Blunting of T-cell-mediated immune response			
	Organotypic co-culture	Collagen IV, laminin, alpha-6 integrin	Disrupted expression	Basement membrane and DJ proteins, disrupted by macrophage invasion	Indirect immuno-fluorescence and zymography	IL-4	[195]
		F4/80, CD-206, Lvye-1	CD-206 and Lvye-1 detected	Macrophage polarization markers (M2 polarization by IL-4)			
	HSE with SCC 12 cell line derived from head and neck SCC	Cytokeratin-7, E-cadherin, Laminin	Downregulated	Differentiation markers	IHC	_	[196]
		Ki-67, MMP-2	Present in invading basal cells	Proliferation and invasion markers			
		P-cadherin, Plankophilin 1	Upregulated	SCC-specific markers			
Hair follicle	3D heterotypic spheroids with HaCaT, DP, and HDF cell lines	BMPs (2,4, and 6), Noggin, Wnt5A	Enhanced expression as compared to 2D culture	Compartmentalization of DP and dermal cells by keratinocytes	qPCR	_	[197]
	3D spheroids by hanging drop cultures	ALP, a-SMA, NCAM	Increased expression in spheroid culture	Hair inductive markers for producing inductive DP cell cultures	qRT-PCR, IF, Western blot	_	[198]
	3D DP spheroids	FN, SPARC, VCAN	Enhanced expression as compared to 2D culture		RT-PCR, IF	_	[199]
Bacterial infections	HSE	IL-8, IL-1α, and IL-1β	Upregulated after 48 h post-infection	Inflammatory cytokines	qPCR, ELISA	*A.baumanii* strain	[200]
	Labskin-HSE	S100 proteins, β-defensin 4A, Elafin, Ubiquitin	Upregulated	Human proteins produced as part of	LESA MS	*S. aureus*, *K. pneumoniae*, and *P. aeruginosa* strains	[201]

(continued)

Table 22.4 (continued)

Human skin diseases	Type of model and skin cells	Biomarkers	Regulation with disease	Description and mechanism	Method of detection	Inducers	Reference
				defence mechanism against infection			
		S. aureus: δ-hemolysin, modulin α3		Bacterial proteins that damage epidermal cells			
		K. pneumoniae: HU-α					
		P. aeruginosa: HU-β					
Fungal infections	Episkin®	Hyphae fragments	Detected	Indicate infection of fungus	H&E staining, PAS staining	*Trichophyton rubrum*, dose of 400 conidia	[202]
	RHE	Tr 18S rDNA			qPCR, H&E staining,	*T. rubrum, Arthroderma benhamiae*	[203]
				Cell viability assay to measure effect of infection on epidermis	MTT assay		
Burns	HSE			Cell viability assay	MTT assay, RNA staining	Contact burn induction with copper device	[204]
		Hsp47	Downregulated in burnt skin	Fibroblast marker that indicates would healing, thus only detected when healing process begins in culture	IHC		
		K17, SKALP	Detected in neoepidermis	Epidermal activation and Stress markers, seen in neoepidermis upon wound healing			
		Collagen 4, laminin	Absent in burn wound	Basement membrane proteins			
		CD31, α-SMA	Detected	Blood vessel and myofibroblast markers; CD31 detected in wound and a-SMA only in the vessels			

Aging	Aged HSE (Senoskin)	SA-β-Gal	Upregulated	A senescence marker is detected in aged fibroblasts	SA-β-Gal staining	H_2O_2, senescent fibroblasts	[205]
		barrier function	Decreased	Hallmark senescence indicator	Biotin permeability assay		
		IL-6, IL-1α, GmCSF	Upregulated	Senescent-associated secretory phenotype	ELISA		
	HSE	Collagen IV/VII, Ki-67, filaggrin, involucrin, loricrin, transglutaminase 1, β-catenin, CD44 (hyaluronan receptor), p16	Downregulated	Parameters of epidermal thickness	IHC		[206]
Photodegradation	HSE	Fibroblasts	_	Apoptosis	TUNEL staining	UVA irradiation	[207]
		LOR	Reduced	Subcellular redistribution due to irradiation	IHC		
		MMP1 (Interstitial collagenase)	Increased	indicates dermal damage	ELISA		
	Phenion® Full-thickness model	GPX1, GSR,	Upregulation at early recovery	Non-enzymatic regulation against ROS formation	qRT-PCR, immuno-fluorescence		[208]
		MMP1, MMP9	Upregulation	Specific markers of UVA damage			
		Involucrin (IVL)	Upregulation only at early recovery times	Indicator of an adaptive mechanism against UVA by increasing SC thickness, but no increase on cumulative exposure			
		IL-10	Dose-dependent regulation	Immuno-suppression and photo allergy mediation			
		Elastin, Collagen-1, fibrillin-1	Upregulation on acute exposure and	Initial upregulation is an indicator of defence mechanisms, long-term			

(continued)

Table 22.4 (continued)

Human skin diseases	Type of model and skin cells	Biomarkers	Regulation with disease	Description and mechanism	Method of detection	Inducers	Reference
			downregulation on cumulative exposure	downregulation is due to damage			
	HSE- DED	IL-6, IL-8	Upregulated	Central cytokines mediating local immune responses	ELISA		[72]
		p53	Detected	Suppression of UV-induced oncogenesis	IHC		
		CPDs	Detected	Specific marker of UVB damage, show immunoreactivity in basal cells			
	EpiDerm™ EPI-200	MMP-1, MMP-3, MMP-7, MMP-9	Upregulated	Formation of ROS	qRT-PCR, IHC		[209]
Particulate matter	SkinEthic™	Lactate dehydrogenase			Colourimetric MMT	PM 0.3–2.5	[210]
		IL-1α, IL-8			ELISA		
		MMP-1, MMP-3		only 2 out of 1 to 13 MMps modulated	Multiplex panel with Luminex® technology		
		4-HNE	Upregulated	lipid peroxidation product	IHC		
		HMOX1, MT1G, and MT1E		Oxidative stress genes	qRT-PCR		

Abbreviations: *FLG* filaggrin, *LOR* loricrin, *CA2* carbonic anhydrase 2, *NELL2* neural epidermal growth factor like2, *IHC* immunohistochemical staining, *TSLP* thymic stromal lymphopoietin, *RHPE* reconstructed human pigmented epidermis, *TGFβ* transforming growth factor β, *(MCP)-1* monocyte chemo-attractant protein, (SPARC) Secreted protein acidic and rich in cysteine, *(LESA) MS* liquid extraction surface analysis MS, *(H and E)* Haematoxylin and Eosin staining, *(PAS)* periodic acid-Schiff staining, *COL1A1* collagen type I A1, *COL7A1* collagen type VII A1, *CCND1* cyclin D1, *DCN* decorin, *ELN* elastin, *FBN1* fibrillin 1, *GPX1* glutathione peroxidase 1, *GSR* glutathione reductase, *IL1A* interleukin 1-a, *IL10* interleukin 10, *ITGB1* integrin b-1, *IVL* involucrin, *MMP1* matrix metalloproteinase 1, *MMP9* matrix metalloproteinase-9, *CPDs* cyclopyrimidine dimers, *4-HNE* 4-hydroxynonenal, *GmCSF* granulocyte macrophage colony-stimulating factor

potential to support prolonged cell viability [19] and pumpless movement of fluids that is recirculated using a rocking platform [20]. However, none of these devices have mimicked the substantial 3D complexity of human skin. Wang et al. developed a novel microfluidic device that replicated the full-thickness human skin and enabled thorough evaluation of permeation and toxicity of drug molecules. The device showed improved epidermal differentiation and resulted in robust dermo-epidermal junctions and enhanced barrier functions [21].

22.1.2 High-Throughput Screening

In the field of biological sciences, the data obtained from samples being evaluated in a single experiment are crucial, especially when many variables are being tested. High-throughput models or systems allow researchers to evaluate hundreds of samples within a short time. High-throughput cell biology has recently been in the spotlight in the areas of capturing cell images, performing cytometry, in drug discovery, for identifying diseased or malfunctioning cell populations, for determining gene expression microarrays, etc. In higher organisms, any response of a body depends on many factors at the level of tissues, cells, proteins, and genes, to name a few. However, the major limitation with 2D cell cultures was low throughput and their inability to represent the organ architectures under in vitro conditions. Thus, scientists started emphasizing on the use of 3D cell culture systems and devices like SOCs, which not only better represent the in vivo skin, but also allowed investigations related to the effect of biochemical and mechanical cues on skin cells.

Intertwining of microfluidics and microfabrication techniques has expanded the field of 3D culture to allow robust manufacturing and fabrication of several 3D skin structures, as may be required during drug development. Monitoring toxicity or efficacy of multiple drugs or using the device as biosensors for pharmaceutical compounds has enhanced the need, demand, and importance of such devices [22, 23]. SOCs have enabled parallel, high-throughput monitoring of chemicals, evaluation of drugs, and real-time biosensing using cell lines. Such models have helped in revealing the mode of action of drugs and have enabled rapid prediction of their efficacy. The endless possibilities of combining other OOCs with SOCs will enable evaluation of the effect of tropical drugs not only on skin, but also on other organs.

22.1.3 Diseased Human Skin Models

The development of healthy skin tissue models is important for applications in many areas of basic research. However, applied research largely relies on availability of diseased skin models, such as models for fibrosis and cancers, etc. to assess the effect of drugs on the human skin. Currently, pre-clinical research is largely dependent on animal models. However, often these fail to successfully predict the outcomes [24] in human skin due to differences in immunity and disease pathology, as compared to

the latter [25]. Here, SOC may be employed to model various skin diseases due to their accurate replication of skin disease conditions and ability to manipulate experimental variables.

A simplistic model was developed to measure the efficacy of dexamethasone, a therapeutic drug for decreasing tumour necrosis factor (TNF-α), for mitigating inflammation and oedema [26]. Further, Ramadan and Ting created an immune competent keratinocyte-on-chip model to demonstrate the effect of lipopolysaccharide and UV radiations [27].

22.2 What Is Skin-on-a-Chip (SOC)?

Skin-on-chip (SOC) is a cell-based in vitro model combining microfabricated culture models to mimic structure and function of the skin. SOC models are a combination of microfabricated culture models and microfluidic technology [28]. Bioprinting, replica moulding, etc. are some of the microfabrication techniques used to fabricate tissue structures [29, 30]. It is possible to control cell shape, position, and 3D arrangement of skin layers to realistically mimic the function of skin [9]. The microfluidic device facilitates a continuous supply of nutrients to the cells and facilitates the discharge of waste products [28]. Small amount of fluids flow within the hollow microchannels of the microdevices, without mixing with each other due to their laminar nature. Hence, small sample volumes can be analysed, and fewer chemicals are consumed. Dynamic fluid behaviours and physical factors such as temperature and mechanical forces can be controlled in the microfluidic platforms [31, 32]. The advanced SOC models include biosensors within them, which enables real-time analysis and measurement of dynamic responses to drugs [33].

22.2.1 Structure and Function of Skin

The composition, architecture, and functioning of human skin should be briefly known to permit the development of relevant SOC models that may closely mimic the in vivo conditions. The skin is a multi-layered structure composed of epidermis, dermis, and hypodermis, as shown in Fig. 22.1. The epidermis is the outermost layer derived from ectoderm and is composed of stratified keratinocytes. It is subdivided into five layers, i.e. stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum [34]. The epidermis is surrounded by an extracellular matrix (ECM) containing carbohydrate polymers and lipids, which is vascular and gets its nourishment from the dermis [35]. The epidermis contains keratinocytes, melanocytes, Langerhans cells, and Merkel cells. Keratinocytes maintain the epidermal structure and provide protection against external damage [36]. Newly formed keratinocytes, in the stratum basale, migrate to the upper layer of the epidermis. The outermost layer or the stratum corneum is composed of corneocytes (terminally differentiated keratinocytes) [37]. Melanocytes are derived from neural crests, found in the basal layer, and synthesize melanin that protects skin from harmful UV

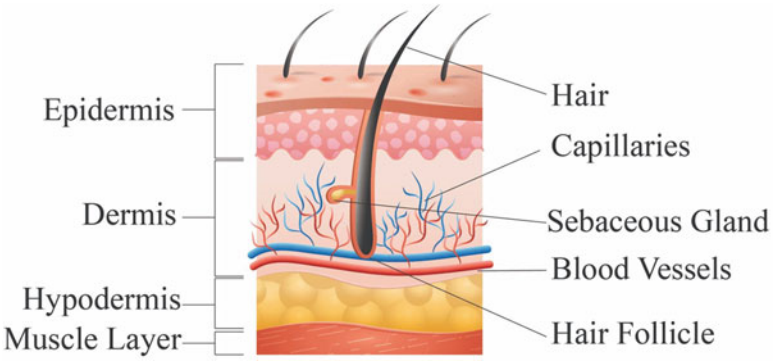

Fig. 22.1 Diagram of Human skin physiology

radiation and pigmentation [38, 39]. Langerhans cells, which are derived from bone marrow, are antigen-presenting cells and play an important role in immunological defence [40]. Merkel cells function as sensory detectors in touch-induced responses [41, 42].

Another important skin layer is the dermis, which imparts mechanical strength and elasticity to the skin. The dermis includes mesenchymal cells, which differentiate into fibroblasts, mast cells, and endothelial cells [43]. Its ECM comprises of proteins like collagen, elastin, and glycosaminoglycan [44, 45]. Collagen provides mechanical strength and flexibility; elastin offers elasticity, while glycosaminoglycans maintain hydration of dermis [46–48]. The dermal fibroblasts synthesize collagen and elastin [49]. Mast cells respond to allergic reactions [50]. Furthermore, dermis houses hair follicles, sweat glands, and sebaceous glands, which are the main barriers and carry out thermoregulation. It also comprises sensory nerves, blood vessels, and lymphatics [45].

The innermost layer is hypodermis, which is composed of adipose tissue, collagen, blood vessels, and nerves. It produces hormones like leptin, which control appetite and metabolic energy [51]. It separates the dermis from the underlying organs. The adipocytes have a role in intercellular communication [19].

22.2.2 Improved Mimicking of Cellular Microenvironment

In vivo cell microenvironment is contributed by cell-cell interactions, cell density, cell-ECM interactions, presence of chemical or secretory molecules, among other factors. In contrast to the traditional cell culture methods, the SOC models provide better insights by accurately controlling several parameters within the device. Also, the device requires very low amounts of samples, cell numbers, and reagents, along with a reduced contamination risk. Lee et al. prepared a SOC model, consisting of human dermal primary fibroblasts enclosed in a 3D collagen hydrogel to represent the dermal layer. HaCaT cells or primary human keratinocytes were seeded on the

top of the collagen-fibroblast layer, which was exposed to an air-liquid interface, in epidermalization medium, to represent the epidermis [52]. Microfluidic SOC devices bring down the cell population to a few hundred cells or even single cells. This allows study of several biological questions based on single-cell resolution or for a cell population, such as capturing perturbations in individual cells, understanding cell locomotion (translocation, protrusion), cell-ECM attachment [53], wound healing dynamics [54], effects of drug treatment, etc. SOCs also reveal complex cellular behaviours like collective cell migration, as shown by Vedel et al., wherein the researchers studied how collective cellular interaction affected cell motility at different densities [55]. Bi-channel microfluidic SOC devices were employed to study the immune components (U937 monocytic cell line) in association with skin cells (HaCaT KC-cell line), as demonstrated by Ramadan and Ting [27].

22.2.3 Evolution of In Vitro Skin Models

Complex and multi-layered 3D models can affect the rate of drug diffusion to mimic the barrier function of human skin, which cannot be studied in 2D cultures [56]. Still, many 3D models are incapable of recapitulating human skin as they lack vasculature, sweat glands, and hair follicles. Also, it is difficult to control chemical gradients and physical factors while studying these 3D models, which has necessitated more advanced fabrication methods [57]. Skin models are developed using primary cells, cell lines, stem cells, and donor-derived cells. The most common approach of in vitro skin development is growing cells in 2D cultures, in plastic flasks/dishes. Although such models are convenient for microscopic analysis, culturing of cells under static conditions affects their proliferation rate, multi-directional growth, morphology, and cell signalling cascades, leading to non-reproducible results [58]. Due to the absence of ECM and limitations related to culturing of single-cell type, the 2D cell models are unable to replicate cell-cell, cell-matrix interactions, nutrient gradients, and hence cannot imitate the in vivo responses of human skin [59].

The complexity of skin can be replicated by seeding keratinocytes on fibrous hydrogels and co-culturing these with dermal fibroblasts. Both natural and synthetic hydrogels, or their mixtures, have been explored for this purpose due to their biocompatibility and mechanical properties. Cells proliferate and differentiate within the hydrogel and display cell-cell and cell-environment interactions, which mirrors the 3D structure of the human tissue. However, such models exhibit challenges for being characterized by microscopy or high-resolution imaging, as the access to the constructed tissue is restricted due to the presence of the hydrogel [60]. It becomes more difficult to study functional aspects of the model due to limited access to the cells entrapped within the polymer matrix. Electrospun or freeze-dried polymers are used to create porous scaffolds for growing the cells in 3D structure. These biodegradable scaffolds allow cells to adhere and proliferate, until they start secreting their own ECM proteins. Decellularized extracellular matrix (dECM), obtained by removing cellular components from the skin tissues (commonly of porcine origin), is also

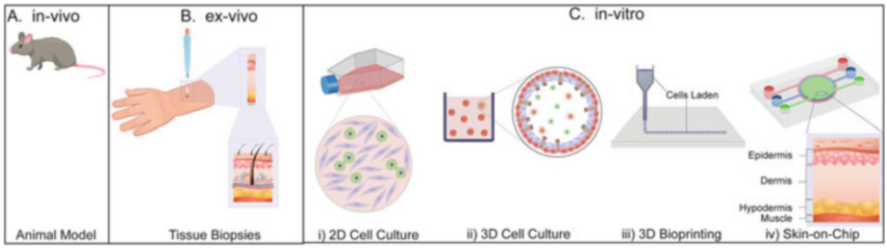

Fig. 22.2 Skin models used for pre-clinical analysis; (**a**) Animal models are commonly used for drug testing; (**b**) Tissue biopsies acquired from human skin; (**c**) In-vitro models used for pre-clinical testing i) 2D cell culture studies where single cells are grown in monolayers; ii) Cells grown in 3D orientation within hydrogel or polymer scaffolds; iii) 3D bioprinting injects cell laden bio-ink in a layer-by-layer manner to create complex tissue structures; iv) Microfluidic skin-on-chip platforms enable mimicking of 3D tissue microenvironment

used to culture human skin cells as it mimics the native human ECM by preserving collagen, glycosaminoglycans, and many growth factors [61]. Although 3D models are more complex than the 2D models, they still lack vascularization, pigmentation, and dermal appendages [28].

Advanced, highly customizable, and computer-aided approach for modelling skin tissue, i.e. 3D bioprinting, involves layer-by-layer and controlled integration of different cell types and growth factors within scaffold materials, as shown in Fig. 22.2 [62, 63]. Further, this approach has been utilized to develop vascularized and pigmented skin [64]. Thus, the bio-printed 3D skin demonstrates a very close comparison to the human skin [65]. Despite its advantages, 3D bioprinting is yet to be widely employed for developing skin models for pre-clinical testing due to highly customized, complex, and expensive instrumentation required for this technology.

The aforementioned models have drawbacks due to which they fail to recapitulate the in vivo conditions, as stated in Table 22.1. Most of these models lack mechanical cues, such as shear stress and strain, which impact in vivo cellular regulation. The SOC models are fabricated by combining microfabrication techniques and microfluidic systems which offer mechanical properties, continuous medium supply, easy access to the cultured tissue, and application of shear forces [32]. While developing microfluidic SOC models, it is very important to take into consideration the major factors such as epidermal barrier which prevents the water loss and protects against UV, harmful chemicals, microbes, and dermal elasticity of human skin. Also, the cultured tissues should have a continuous supply of gases and should allow timely removal of waste materials, as the native skin is a dynamic organ due to the presence of blood vessels [13]. It is important to consider the strength of skin barrier while developing a skin model to test drug efficacy. The mechanical forces that act on the cells should be replicated as the mechanical signals are converted into biochemical responses by a phenomenon called mechano-transduction, which can affect characteristics of the skin model [57]. These models can be fabricated using biocompatible, optically transparent materials, such as silicon-based elastomers like polydimethylsiloxane (PDMS) and glass and thermoplastic polymers such as

Table 22.5 Advantages and disadvantages of various in vivo, ex-vivo, and in vitro approaches used for studying skin

	Approach	Advantages	Disadvantages
In vivo	Animal model	Systemic evaluation can be done	Show interspecific variability Ethical issues
Ex vivo	Tissue biopsy	All the required and suitable cells are present	Invasive models Low availability
In vitro	2D cell culture models	Well established models Easy to handle	Static models Lack tissue complexity
	3D cell culture models	Cell-cell and cell-ECM interactions are possible The 3D architecture of tissue	Inefficient nutrient transport
	3D Bioprinting	High-throughput system Chemical gradient achieved	Lack vasculature Challenges in fabricating cells with materials
	Skin-on-chip	Customized and controlled microenvironment Continuous supply of nutrient	Highly complex Difficult to standardize and scale-up

polystyrene (PS), polycarbonate (PC), and polymethyl methacrylate (PMMA) [66]. Transwell holders/thin membranes used within the microfluidic devices enable compartmentalization of the co-cultured cells or cells and media containing drugs and/or cytokines (Table 22.5).

22.2.4 Types and Variations in In Vitro Skin Models

Researchers have used advanced biomaterials to construct 3D skin models, like reconstructed human epidermis (RHE) [67, 68], full-thickness skin models [69], and full-thickness skin models with additional cell types [70, 71] to study the efficacy and penetration of drugs and conduct elaborate investigations related to skin biology.

22.2.4.1 Reconstructed Human Epidermis (RHE)

Construction of an epidermal equivalent should take into consideration layers, such as stratum corneum, stratum basale, stratum lucidum, stratum granulosum, and stratum spinosum. Human keratinocytes were cultured over polycarbonate membranes, at an air-liquid interface. The keratinocytes matured into cornified cells and were used to study responses to mutagenic UVB. The UVB irradiated skin constructs showed activation of apoptotic pathways, accumulation of p53, and secretion of inflammatory cytokines [72]. Models such as Episkin® and EpiDerm™ were fabricated by culturing human keratinocytes within a collagen-based matrix. The Episkin® model is suitable to identify the possible weak phototoxic substances. When the model was exposed to 6-methylcoumarin and ofloxacin (weak phototoxic substances) in the presence of UVA, an increase in cell death and release of IL-1α were observed, indicating the phototoxicity of the substances [73]. The Episkin® model was exposed to various surfactants like Polyoxyethylenesorbitan mono

monolaureate (Tween 20) and Sodium dodecyl sulphate (SDS). The studies showed that due to the impaired barrier function, the concentration of the surfactant required to cause irritation in the model was lesser than required in vivo. Some studies showed that both the models are more permeable to caffeine, α-tocopherol, and mannitol than normal human skin [74, 75]. Level of IL-1α mRNA was measured to determine the irritancy level of the EpiDerm™ model due to sodium lauryl sulphate, which differed from that of the excised human skin [76]. Modified skin model, SkinEthic, is being used to study irritation on skin due to sodium dodecyl sulphate, heptanoic acid spiked in polar solvents, sodium chloride solution or phosphate buffer saline, lactic acid and non-polar solvent, sesame oil, etc. SkinEthic™RHE model was able to detect the irritant activity and decrease in cell viability was observed [68]. A wide range of polar penetrants like terbinafine, clotrimazole, hydrocortisone, and salicylic acid were used to assess the permeability of the model compared to human skin. The studies showed that the SkinEthic model lacks sufficient barrier function [77]. Due to inefficient barrier function, the above-mentioned models may lead to false positive data in transport studies. Skin models, like MelanoDerm™, constructed using keratinocytes and melanocytes were co-cultured with fibroblasts from the melasma lesion which secrete nerve growth factor NGF-β and lead to increased pigmentation in the model than a negative control. Such models have been used to assess the efficacy of pharmaceutical agents that regulate skin pigmentation [76]. The permeation coefficients obtained through permeability studies in these models, using caffeine and testosterone, differed from those measured across human skin, thus calling for an improvization in the RHE models [78].

22.2.4.2 Full-Thickness Skin Models (FT)

Advanced RHE models include dermal equivalents along with the epidermal layer, wherein human dermal fibroblasts are cultured in collagen type I, while epidermal keratinocytes are cultured over the dermal layer at an air-liquid interface. These models include EpiDerm-FT, Advanced-Skin-Test 2000 (AST-2000), and Strata Test [28, 79]. Wound healing requires interaction between keratinocytes and fibroblasts. Keratinocytes proliferate in presence of growth factors secreted by the fibroblasts and in turn release cytokines for the proliferation of fibroblasts. Serum contains various proteins, cytokines, and growth factors that are used to maintain the dermal layer [80, 81]. The full-thickness models display relevant barrier functions, as compared to the RHE models, when treated with corrosive substances. The FT models are better at mimicking the native skin tissue, as compared to the RHE models, and result in a higher cell viability [82]. The lipid composition of FT models has the same arrangement as the normal human skin, but the lipids are more abundant as compared to the latter [83]. EpiDerm-FT model was subjected to saturated aliphatic hydrocarbons to understand the skin irritation effects. The chemical treatment of 24 h showed slightly disturbed stratum corneum and increased level of inflammatory markers like IL-1α, IL-6, and IL-8. The EpiDerm-FT serves as an excellent model to evaluate the irritation due to chemicals [84]. AST-2000 was used to evaluate the phototoxicity properties of substances like promethazine and chlorpromazine. The skin model showed increased histological damage, reduced cell

viability, and increased level of IL-8 and PgE_2. AST-2000 is a beneficial tool to evaluate the phototoxicity of concerned substances [85]. Strata Test shows the presence of well-defined epidermal compartment, but reduced barrier function. Cytotoxicity of sodium dodecyl sulphate was assessed by determining the cell viability and quantifying the release of IL-1α. Effect of UV light on the model was also evaluated which resulted in increased intracellular ROS levels. The level of ROS formation was observed to be reduced when the skin construct was pre-treated with UVA/UVB sunscreen [86]. These FT models recapitulate the native human skin. However, they need to be improved with respect to their lipid composition and other features, like the skin appendages.

22.2.4.3 Full-Thickness (FT) Skin Models with Additional Cell Types

Full-thickness models developed by incorporating skin features like pigmentation, sebaceous glands, sweat glands and blood vessels, nerves, and immune cells can mimic the human skin. Melanocytes (pigmentation) cells were co-cultured with keratinocytes and fibroblast cells to examine vitiligo disease. This model was used to analyse normal and vitiligo melanocytes and to check the response of melanocytes against melanogenic stimulators and inhibitors. When the model was exposed to stimulators like Latanoprost and Stem cell factor, the tyrosinase activity, cell proliferation, and melanin content increased, but the same model when treated with melanogenic inhibitors like Kojic acid and Hydroquinone, the same parameters were observed to be decreased. This model can be used to assess limited number of compounds [87]. Incorporation of Langerhans cells in skin models has helped researchers to study the immunological responses of the skin to allergens, such as nickel sulfate, resorcinol, and cinnamaldehyde, and irritants like Triton X-100, SDS and Tween-80, which resulted in the migration of Langerhan cells (LC) into the dermal layer. Anti-CXCL12 antibody and Anti-CCL5 antibody blocked the migration of LC due to allergen and irritant, respectively. This model is a useful tool to study the human LC activation and migration upon exposure to chemicals and screening novel drugs therapeutics [88]. Chau et al. developed a 3D skin equivalent using monocyte-derived dendritic cells that were cultured along with epidermal and dermal layers in an agarose-fibronectin gel, to demonstrate the immune response towards allergens, in case of diseases like dermatitis. The model was sensitized using dinitrochlorobenzene (DNCB). No significant difference was observed in the cytokine profile, but dendritic cells showed migration into the dermal layer and responsiveness by upregulation of expression of CD86 and HLA-DR. The model is useful to study the sensitization mechanism of skin and evaluating the allergenicity [89]. A 3D skin equivalent developed by Van den Bogaard et al. using keratinocytes and CD4+ T-cells was used to study inflammatory diseases like psoriasis, by analysing the expression of proteins associated with psoriasis like hBD2, KRT16, and elafin and pro-inflammatory cytokines IL6, IL8, IL23. Downregulation of psoriasis-associated protein was observed when the culture medium of the model was treated with all-trans-retinoic acid (ATRA) or Cyclosporin (CsA). This model is suitable to screen anti-inflammatory pharmaceutical agents [90]. Epidermal differentiation was improved when the FT models were constructed

using the combination of hypodermis and adipocytes [91, 92]. Skin constructs can be fabricated along with blood vessels for developing long-lasting models due to a continuous supply of nutrients and oxygen. Human vein endothelial cells (HUVECs) and induced pluripotent stem cells (iPSCs) were patterned using 3D bioprinting technology to develop vascularized skin equivalents. The formation of endothelial barrier was demonstrated using fluorescein isothiocyanate (FITC)- Dextran. The model is suitable to perform studies related to the delivery path of therapeutic agents [93]. However, successful incorporation of hair follicles in full-thickness skin models is still awaited.

22.2.4.4 Skin-on-a-Chip (SOC) Models

Skin-on-Chip (SOC) model includes culturing skin tissues with the microfluidic system incorporated within it. Dynamic SOC models have been developed to reflect the mechanical stress conditions faced by the cells and perfusion systems to maintain the cultures for a longer duration. The SOC model comprising of epidermal, dermal, and subcutaneous layers and exhibiting an improved cell viability and tissue architecture enabled researchers to conduct drug screening and toxicology studies. Wufuer et al. developed a microfluidic skin-on-chip model to study inflammation with inserted monolayers of keratinocytes (KCs), fibroblasts (Fbs), and vein endothelial cells. Dexamethasone (Dex) is a well-known drug to treat inflammation. The developed model with induced inflammation was used to demonstrate the reduction in inflammatory cytokines IL-1β, IL-6, and IL-8 after the treatment of Dex [94]. Immortalized keratinocytes, such as HPV-16, HPV-18 [95], HaCaT cells [96], and hTERT, immortalized foreskin keratinocytes and fibroblasts [97]; fibroblast cell lines like MRC-5 [98] have been employed to fabricate SOC models. Atac et al. demonstrated the advantages of microfluidic culture, such as prolonged culture duration and higher cellular viability [99]. A pumpless microfluidic model developed for drug testing, by Abaci et al., circulated the culture medium through the skin equivalent placed within the device, with the help of a rocking platform. The developed model was tested using an anticancer drug, doxorubicin. The treated sample showed no proliferation of basal keratinocytes as compared with treated samples [20]. Ramadan and Ting set up a microfluidic device for co-culturing keratinocytes and dendritic cells, to establish enhanced barrier integrity as compared to static cultures and for mimicking the immune responses of the skin. A moderate increase in the expression of pro-inflammatory cytokines, IL-6 and IL-1β, was observed when the model was treated with lipopolysaccharide (LPS). The model can potentially be used to study skin sensitization and immune response [27]. Mori, Morimoto, and Takeuchi designed a skin model with vascular channels to study the diffusion of chemical components. Percutaneous absorption was monitored using test molecules, caffeine, and isosorbide dinitrate (ISDN). The studies demonstrated that the developed model mimics the in vivo endothelial function and vascular endothelial growth factor (VEGF) controls the vascular channel permeability [100]. Future approaches involving insertion of several cell types to develop healthy and diseased skin models will benefit researchers to deduce the pathways of infections and to develop novel treatments.

22.2.5 Minimal Requirements for SOC Modelling

Conceptually, the development of SOC models requires consideration of multiple important factors. They usually comprise of one or more than one skin cell/tissue culture chambers, which are efficient in maintaining air-liquid (AL) interface and well connected with direct/indirect perfusion system that circulates the medium through the lower layers of the chamber/s and circulate fresh nutrient medium across the membrane for diffusion of sterile medium in the culture chamber for the growth of 3D skin culture. Size of skin cell (25–40 μm^2)/tissue culture chamber and capillary for appropriate perfusion and flow rate of the nutrient medium across the chamber/s or whole device are crucial factors. Migration rate of cells before choosing the cell type, e.g. fibroblasts (Fbs) migrate up to 200 mm/day, is important.

The human skin epidermis contains keratinocytes (95%), with remaining 5% composed of melanocytes, inflammatory cells, Langerhans, and Merkel-Ranvier cells (tactile epithelial cells) [101]. Skin dermis is primarily composed of Fbs, adipocytes, macrophages as well as blood vessels and nerve receptors [102, 103]. The mean thickness of human skin epidermis ranges from 76.9 ± 26.2 to 267.4 ± 120.6 μm, whereas for dermis it ranges from 2115 ± 946.4 to 5888 ± 2422.3 μm [103]. Cell type selection and cell seeding density are important depending on the model being engineered, e.g. full-thickness model (FTM), reconstructed human epidermal skin model (RHE), reconstructed human dermal skin model (RHD), and skin disease model (SDM) on perfusion-based microfluidic chip.

General cellular requirements and their maintenance throughout the developmental stages, including culture media, growth factors, CO_2, O_2, pH, osmolarity, temperature, pressure, and humidity, are necessary for SOC development. Scaffold selection and functional gradient preparation are important for obtaining appropriate cell morphology and mechanical strength. The device materials and fabrication techniques should be compatible with skin cells and amenable for long-term use. SOC model should be made so that it can satisfy the biomechanical and physiochemical skin tissue characteristics and mimic the in vivo microenvironment and functions of a healthy/diseased human skin closely. Two major approaches have been utilized for designing SOC devices that replicate the human skin, viz., (1) in situ skin-on-a-chip (SOC) which involves 3D culturing of skin cells in chip, leading to the development of epidermal or dermal or full-thickness epidermal-dermal skin in vitro as required by the tissue engineer; (2) transferred skin-on-a-chips (SOC), i.e. introduction of human skin equivalent (HSE) or skin sections/fragments from biopsy samples directly in the chip.

The general procedure for 3D skin culture development in a microfluidic system involves several steps. Abaci and co-workers extracted fibroblasts and keratinocytes from neonatal skin tissue by organotypic culture [104, 105], which is the primary and most efficient method for primary skin culture. Isolated and passaged (passage no. 3 to passage no. 9) fibroblasts were mixed with different kinds of dermal matrices, such as type I collagen containing materials. The mixture was cultured on a porous membrane like polyethylene terephthalate, polycarbonate, cellulose, etc.

Post-polymerization of this dermal matrix, usually the culture is maintained for 3–7 days. Thereafter, the keratinocytes were seeded on top of the fibroblasts-collagen-based matrix and incubated for a minimum of 7 days, along with submerging into epidermalization medium [104]. The 3D construct was subsequently exposed to cornification medium [104], at a reduced level, and subjected to air-liquid (AL) interface for 7–28 days [20]. Thereafter, the full-thickness skin model was harvested for characterization and validation.

22.3 Components and Development of Skin-on-a-Chip (SOC) Devices

The SOC is an on-chip live skin tissue, cultured within the microfluidic device, that facilitates continuous medium perfusion through the developed tissue. The basic components of microfluidic SOC model include biomaterial, scaffolds, skin cells, reagents, model ingredients, and general materials. Epidermal/dermal/epidermal-dermal or 3D skin reconstructs like FT skin model have been created using various microfabrication techniques like lithography, 3D printing, laser cutting and CNC micro-milling, etc.

22.3.1 Biomaterials and Scaffolds

Various parameters affect modelling of skin microenvironment using a biomaterial scaffold. The biomaterials employed in these models should be safe, non-toxic, biocompatible, and should not elicit an immune response. They should be porous enough to enable proper cell migration and cell proliferation. The degradation rate of biomaterials should be inversely proportional to the regeneration of skin cells. The toxic compound released after degradation should be easily removable [106–108]. A skin scaffold is a supporting 3D structure that directs and supports cellular activities, such as proliferation, differentiation, adhesion, expression, and localization, leading to the growth and development of functional skin tissue [109, 110]. According to the American National Institute of Health (NIH), a biomaterial is defined as a combination of materials or components, including substances of natural or synthetic origin, and except drugs, which are engineered to be used for a certain period, to partially or wholly replace or augment any tissue, organ, or a function of a body, to uphold or support the requirements of human body [111]. Scaffolds for skin engineering should be able to support different skin cell types.

In general, scaffolds for skin tissue engineering should offer properties [111–113] like bio and cyto-compatibility, which depends on scaffold's surface charge, porosity, topography, exposed chemical groups, moisture retention ability, and rate of water vapour transmission. Moreover, the scaffolds should support exchange of metabolites, nutrients, and gases via well-connected pores (in case of in vitro skin, the pore size should be around 5 mm) [113–115]. Suitable composition and continuous and non-continuous structural and physico-chemical gradients are necessary to

provide appropriate environment for several skin cell types and layers, which enables proper formation of new skin tissue having desirable barrier integrity, cell migration rate, aquatic fluidity, remodelling, integration, and improved angiogenesis/vascularization [116, 117].

Other properties such as hemocompatibility, non-immunogenicity, i.e. non-inflammatory nature, non-allergenicity, non-antigenicity, non-thrombogenicity and absence of fibrotic overgrowth, non-pyrogenicity, non-carcinogenicity, non-cytotoxicity, non-genotoxicity, and non-mutagenicity, are also important. Biodegradability and biomechanical stability are crucial as they govern the cell viability, cellular phenotype, differentiation, cell-matrix interactions, and size of focal adhesions, ultimately sustaining overall cellular mechanotransduction and subsequent cellular behaviour in the system. Finally, scaffolds employed for supporting skin tissue should have the ability to promote self-repair and exhibit a long shelf-life, suitable physico-chemical characteristics, and economic feasibility.

Accordingly, scaffolds used for SOC development can be categorized into three different groups [117] based on:

- The type of biomaterials as natural, synthetic, and composites
- Their structure as porous, fibrous, decellularized, hydrogels, and microspheres
- Different skin layers such as epidermal, dermal, epidermal-dermal scaffolds

Scaffolds/biomaterials which can be used for developing artificial skin equivalents or SOCs comprise of a single or combination of polymers, such as collagen, gelatin, alginate, polycaprolactone, elastin, poly (ethylene glycol), fibrin, hyaluronic acid, chitosan, silk, poly (vinyl alcohol), polylactic acid, hydrogels, etc. Collagen is the most abundant component of skin and is responsible for providing structural support and mechanical strength. It is the most widely used to develop skin alternatives [118]. Mechanical properties of scaffolds can be improved by techniques such as chemical crosslinking, co-polymerization, and blending with different cellular proteins, like collagen type IV, fibronectin, laminin, entactin, perlecan (heparan sulfate proteoglycan), and glycosaminoglycans (GAGs). Matrigel® is the most widely used commercial polymer matrix that has been used to develop artificial skin [113, 114, 117].

22.3.2 Material of Construction for SOC Microfluidic Chips

Fabrication of microfluidic chips requires biocompatible materials, such as poly di-methyl siloxane (PDMS) or polyurethane, etc. Membrane filters (0.1–0.2 μm pore size), like cellulose, polycarbonate, polyester, polyethylene terephthalate (PET), syringes of different capacities, components to maintain perfusion conditions around the tissue culture chamber/s, inlet/s, outlet/s, reservoirs for nutrient media replenishment and circulation, push-pull pumps (if perfusion-based microfluidic system is

employed), and cell culture inserts, can all be used for the development of SOCs, either individually or in combination [99, 110].

The base materials are usually polymers or hydrogels, which are processed using standard micropatterning and soft lithography techniques, such as hot-embossing or injection moulding [18, 119–123]. These devices can fit any budget because of their cost-effectiveness, and their ability to be fabricated using diverse inexpensive techniques and materials like PDMS, consume lesser amount of media, and avoid the unnecessary patient trials. The commercial cell diffusion devices costs around 440 USD which can be easily replaced by PDMS-based microfluidic devices costing 1 USD (material cost) [13]. When it comes to fabrication, selecting hydrogel materials for SOC devices allows introduction of biomimetic properties and mechanical properties of the substrate as their surface can be coated with peptides or proteins for promoting cell adhesion [123, 124]. Another advantage of hydrogels is that they can undergo triggered biodegradation processes, which can turn into natural ECM proteins like collagen, gelatin, or fibrin [125–127]. Simple freeze-drying methods can be used to make porous scaffolds for better mimicking the skin structure. Also, electrospinning may be used as an alternative method to produce porous skin substitutes, with architecture matching with the native ECM. These porous structures help cells to better proliferate and adhere [8, 128, 129].

In recent days, 3D printing has enabled high versatility in designing of microfluidic skin constructs, with custom layouts to print layers of desired thickness, by a layer-by-layer approach. Polymeric materials, such as polydimethylsiloxane (PDMS), are majorly used by stacking two PDMS slabs with a polycarbonate porous membrane in between. PDMS devices allow integration of microfluidics as both, static and perfusion modes, at room temperature. The easy, diverse, and customizable fabrication techniques and affordability have rendered SOC as a better and full proof platform for research related to in vitro skin.

22.3.3 Skin Cells and Reagents

Reagents like skin cells/tissue-specific growth factors like fibroblasts growth factors (FGFs), keratinocyte differentiation factors, and importantly culture media such as Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS) or 10% fetal bovine serum (FBS), calcium, growth factors (GFs), and cell adhesion molecules like fibronectin (if required), are crucial for maintaining adequate growth and cell proliferation. Along with 3D scaffolds/biomaterials, as described in Sect. 22.4.1, skin cells like fibroblasts, keratinocytes, melanocytes, immune cells, and adipocytes form the imperative components of microfluidic SOC devices. Further, different enzymatic reagents like dispase II (1 g/L), trypsin-ethylene diamine tetra acetic acid (EDTA), accutase, and collagenase are used to dissociate or isolate cells from primary skin tissue to provide the desired cells or 3D/2D cultures to enable their accurate characterization [130].

22.3.4 Microfabrication of SOC Device and Its Functionality

In 1998, Whitesides introduced the soft lithography technique for fabricating microfluidic devices [131]. These devices are primarily fabricated using elastomeric materials, such as polydimethylsiloxane (PDMS) fabrication [132]. Soft lithography has been widely used for designing microfluidic SOC devices. The general and most applied fabrication procedure involves (1) mixing and degassing of PDMS for well molding, (2) fabrication of the PDMS well, and (3) chip assembly. The wafer master, which is prepared using SU-8 photoresist, silicone elastomer base, and curing agent (for ex, marketed PDMS sylgard 184 A and B respectively), are mixed in the ratio of 10:1 (base:curing agent) weight proportion. Vacuum is applied to remove air bubbles from the PDMS using vacuum dessicator. Followed, the mixture is poured into fabricated replica molds and cured at 60 °C for 6 h. The lower PDMS layer comprises of channel patterns, while the upper layer is without any pattern and is prepared as the medium reservoir space or either directly connected to inlet/s and outlet/s syringes via push-pull pump. Culture and media reservoir chambers are fabricated using biopsy punches. Multiple adjacent overlapping punches are employed to fabricate the reservoir area at the same location on both the sides of upper PDMS layer. A porous membrane (as mentioned in Sect. 22.4.2) is placed in between the PDMS layers, above a glass slide and plasma bonded to enable proper bonding and sealing of the layers as shown in Fig. 22.3a [52].

A custom made, gravity- or syringe-driven microfluidic system is employed for media perfusion, which is directly controlled by a computerized program [52, 133]. In case of bonded perfusion-based microfluidic SOC devices with overlapping punches for reservoirs, it is kept in a dish holder, on a rotating stage that is connected to the computer for controlling the medium flow rate. This is done by automated tilting of the chip angle, at 15° for the desired period, for measuring the geometry and frequency of fluidic channels. After a certain period, it is shifted to the opposite angle, so that culture medium is continuously recirculated [134]. In case of push-pull syringe pumps, the bonded perfusion-based microfluidic SOC devices are well connected with a push-pull automated pump/s (e.g. KD scientific pump instruments) for withdrawal and insertion of media via syringes (e.g. BD plastic syringes with capacity 5 mL, 10 mL, 50 mL, etc.).

Microfabrication of the devices gives a user better control while handling smaller quantity of fluids. The skin cells are cultured on 6, 24, or 96 well plates for various applications, wherein the cell population is seeded at the density of 10^4–10^5 cells per well, depending on the well size. Rinsing or washing the wells cannot be easily done by pipetting and requires multiple pipetting and washes from time-to-time. Introduction of microfluidic perfusion in a SOC device by Warrick et al. resolved this problem and enabled improved sample treatment of the cells [135]. SOCs enable controlled and accurate treatment of skin with chemicals or drugs in a high-throughput experiment. Also, with SOC devices, 14 days of repeated testing is possible, while maintaining the cell viability, Advancement in soft lithography and microfluidics has allowed a precise control over pre-clinical studies, rendering them more robust, high-throughput, and cost-effective [136].

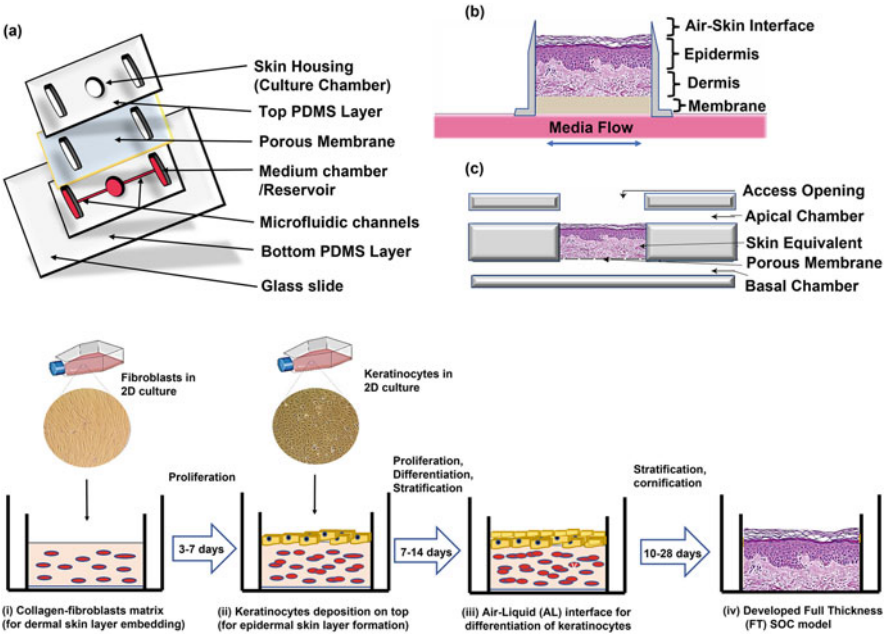

Fig. 22.3 General layout of the microfluidic skin-on-a-chip (SOC) device. (**a**) Different layers of the device, (**b**) culture chamber showing several skin layers, media flow direction, and air liquid (AL) interface (**c**) representation of apical, basal, culture chamber w.r.t membrane and 3D skin (equivalent), (i–iv) different stages in development of full-thickness (FT) SOC model

22.3.5 Characterization of 3D Skin Cultures

22.3.5.1 Morphological Characterization

The human skin has a range of features that govern its biology and responses. In vitro tissue developed within SOC devices can be compared with the human skin using several techniques. Morphological characterization to study the cellular viability and cellular orientation/organization and confirm the in vitro formation of dermal and epidermal skin layers within the culture chamber is one of the basic and primary characterization studies. Phase contrast microscopy, fluorescence (FL) confocal (such as Leica Microsystems laser scanning confocal microscope), and high content scanning microscopy (HCS) such as Perkin Elmer Operetta system, two-photon microscopy, confocal Raman spectroscopy, etc. have been utilized for analysing 3D skin tissue. HCS systems have been employed for understanding the orientation of individual skin cells within the 3D construct, over the depth of 2–4 mm overcoming the limitation of analysing the 3D skin culture up to 300 μm in other analysing systems.

22.3.5.1.1 Cell Viability and Orientation of 3D Skin Cultures

Cellular viability is determined using Calcein acetoxymethyl ester (Calcein AM) and propidium iodide (PI)/ethidium homodimer (EthD-1)-based live-dead staining dyes

mostly. Differential staining with CellTracker™ dyes is used for distinguishing the individual cell types within the 3D skin cultures.

22.3.5.1.1.1 Live and Dead Cell Staining/Cell Viability Studies

Live cells are mostly distinguished by staining with Calcein, formed by the cleavage of cell-permeable and non-FL Calcein AM due to the action of cytoplasmic esterases. The polyanionic dye calcein is well retained within live cells, emitting an intense, uniform green FL (excitation (ex)/emission (em) ~495 nm/~515 nm) [147]. PI and EthD-1 are the popular red-FL nuclear and chromosome counterstain. Since PI/EthD-1 is not permeant to live cells, it is also commonly used to detect dead cells in a population. It binds to DNA by intercalating between the bases, with little or no sequence preference. Once the dye is bound, its FL is enhanced 20–40-folds, producing a bright red fluorescein dead cell with an ex maximum (ex max) at 495 nm and FL em max at 635 nm [148, 149]. Background FL levels are inherently low with this assay technique because the dyes are virtually non-FL before interacting with cells. Imaging is conducted using confocal and HCS systems using an ex/em 495 nm/515 nm for Calcein AM to image the live cells. On the other hand, PI (ex/em, 493 nm/636 nm) and EthD-1 (ex/em, 528 nm/617 nm) are mostly used to image the dead cells [150]. FL images obtained by sequential scanning can be merged. Z-stack imaging can be performed to study the cell viability of 3D cultured skin fibroblasts (Fbs), keratinocytes (KCs), human umbilical vein endothelial cells (HUVECs), and cells/cell lines at different planes throughout the 3D skin construct [89, 130, 151–153].

22.3.5.1.1.2 Differential Staining Using Cell Tracker Dyes

The CellTracker™ dyes on permeation through cell membranes get converted into cell-impermeant, fluorescent reaction products. These dyes are often inherited by the daughter cells in the growing cell population. They are generally used owing to their tracking properties and are observed to retain through three to six cell generations. The dyes are non-toxic and stable in skin cells/cell lines such as human umbilical vein endothelial cells (HUVECs), Fbs, KCs at working concentrations (5–25 μM), and the physiological pH. Chemically, they contain a chloromethyl group that reacts with thiols, probably in a glutathione *S*-transferase-mediated reaction. In most cells, glutathione levels are high (up to 10 mM) and glutathione transferase is ubiquitous. The dye is transformed into a cell-impermeant fluorescent (FL) dye-thioether adducts for analysis by FL microscopy, at dye-specific ex/em wavelengths [154, 155].

CellTracker™ (Green CMFDA), CellTracker™ Blue, and Red CMTPX dyes are mostly used to stain different skin mimicking layers growing in SOC to investigate their proper formation [26].

CellTracker™ Green CMFDA dye imparts green FL at ex/em of 492 nm/517 nm, whileCellTracker™ Blue, Red CMTPX dye exhibits blue, red FL when studied at ex/em of 353 nm/466 nm, 577 nm/602 nm, respectively. The 3D FL images of skin cultures are imaged with confocal and HCS microscopy to characterize different skin cell types and are cultured uniformly over the area of culture chamber forming a

layered structure of distinguished epidermal and dermal human skin regions. FL images obtained by sequential scanning can be merged and Z-stack imaging is usually performed to study the distribution of dermal and epidermal layers within the 3D construct, at different planes in the SOC models [154].

22.3.5.1.2 Histological and Immunohistochemistry (IHC) Analysis

3D skin constructs and cultures developed using SOC devices are further sectioned and analysed using various histological and staining methods to understand the formation and presence of different skin layers. Immunostaining is conducted with skin cell/cellular protein/s-specific antibodies to ascertain the expression of cellular markers in a multi-layered morphology [130, 152]. Skin equivalents need to be tested regarding their similarity with in vivo skin using morphological (architectural) and immunohistochemistry (IHC) (functional) analysis. Histological sections are stained with hematoxylin & Eosin (H&E) for skin layer, Masson's trichrome (MT) for collagen fibres, periodic acid-schiff (PAS) reagent for basement membrane and glycogen, Weigert-Van Fieson (WVF) for elastic fibres, and Fontana-Masson (FM) for melanocytes. IHC is used to localize and stain vimentin (positive marker for dermal Fbs) and pan cytokeratins (CK) CK AE1/CK AE3, CK 34Be12, CK 35Bh11, CK7, CK20, CK10, E-Cadherin, Claudin-1, p63, filaggrin, loricrin (for epidermal KCs), S-100 proteins (melanocytes, dendrites), CD68 (histiocytes), CD34 (endothelium), ki67 (proliferation marker), [21, 89, 130, 152]. These marker patterns are present in human skin in vivo also and are successfully established after identifying similarity in the in vitro developed 3D skin in SOC model.

22.3.5.2 Biophysical Characterization

Skin cells actively sense mechanical forces which play a significant role in guiding cellular functions. Skin cell and tissue responses to mechanical stimuli result in adaptive alterations that regulate its form and function in vivo. When such biophysical changes and cues are absent or erroneous, disarrangements in the structure and function of the skin occur (e.g. during development and metastasis in skin cancer, the biomechanical properties of tumorous cells change) which is a very crucial factor for pre-clinical testing of pharmaceutical drugs and validates healthy SOC and diseased SOC models as an efficient alternative to animal model [156].

Biomechanical and rheological studies, which include (1) determination of young's modulus (E), i.e. modulus of longitudinal elasticity—states relation between stress (σ) and strain (ε) in the skin [157, 158], by dynamic cell stretching systems, using mechanical analyser and tensiometer [159]; (2) high-resolution imaging technique such as multiphoton microscopy (MPM), are commonly used for biophysical characterization of developed in vitro skin cultures in SOC models. The E for healthy human skin generally fluctuates in the range of 1.6 MPa and 0.05 MPa [157, 160]. Cyclic equibiaxial/uniaxial strain capable of achieving optimal change in surface area of the biomimetic 3D skin culture (skin equivalent) at an optimized frequency (in Hz) is induced in SOC using tension-based systems like tensiometer to determine E. It characterizes skin resistance to elastic elongation or changes due to other external factors like temperature and environment [159, 161,

162]. The resulting morphological changes in skin equivalent are assessed at cellular level by MPM. MPM are widely used to investigate developing skin equivalent and human skin, which works on the principle of non-linear excitation (ex) of endogenous or exogenous FL molecules. It has wide range of application in skin cancer diagnosis [163], skin ageing [164], drug monitoring [165], etc.

22.3.5.3 Functional Characterization

Functional characteristics of the 3D skin cultures developed within the SOC devices are determined qualitatively and quantitatively to confirm and measure barrier integrity before pre-clinical testing of skin-related medicines or drugs. Transepithelial/transendothelial electrical resistance (TEER) is the most widely used quantitative method to measure the integrity of tight junction dynamics in SOC models. TEER values are strong indicators of the cellular barrier integrity and permeability before evaluation of chemical/drug transport. It is generally based on measuring ohmic resistance or impedance across a wide range of frequency. It can be performed in real time without any cellular damage. For successful use of SOC for drug absorption prediction, it depends on how closely the SOC mimics the characteristics of in vivo barrier integrity [57, 144, 166, 167]. Instruments like Voltammeter are commercially available measurement system or are also inbuilt in the microfluidic SOC devices as biosensors [21]. Advantages of TEER analysis are its non-invasive approach and real-time live cell growth and differentiation stages monitoring [168].

Qualitatively, it can also be determined using freeze fracture electron microscopy or confocal, HCS FL microscopy of transmembrane fibrils, immunostaining of proteins that represents characteristic of tight junction such as occluding, ZO-1 and ZO-2 [152, 166]. Loss of skin epithelial barrier function can also be quantified by the extravasation of fluorescently tagged molecules such as fluorescein isothiocyanate (FITC)—conjugated dextran (70 kDa, FITC-dextran), across the 3D skin culture in SOC. This is measured by spectrophotometry using microplate reader and FL spectroscopy by allowing diffusion of FITC-dextran which shows ex/em 490 nm/520 nm [21, 169–171]. Table 22.2 states the list of characterization techniques that have been used to develop various microfluidic SOC models, till date.

22.3.6 Validation Using Skin-Related Pharmaceutical Products and Tests

Human skin is the largest organ and highly accessible regions of the body for topical and transdermal cosmetics or various pharmaceutical product deliveries. Microfluidic SOC model to check the therapeutic efficacy and unwanted side effects of these products is important to production. It is very important to ascertain whether the SOC models are efficiently mimicking the human skin, for efficient pre-clinical evaluation of drugs, formulations, and significant applied alternative to animal models like rodents' pharmaceutical research and industry. To evaluate its correlation with in vivo skin, SOC is developed by Lukacs et al. for in vitro/in vivo

monitoring of hydrophilic model drug, caffeine tested in a vaseline-based cream formulation. The drug penetration is measured in both native and 3D skin reconstruct using SOC devices, compared with franz diffusion cell system and transdermal micro-dialysis (TdMD) has been successfully demonstrated to validate SOC efficacy and resemblance to human skin tissue [172]. Quinidine and erythromycin drug adsorptions are also compared and validated using both TdMD in rat skin and microfluidic SOC model [173]. The study by Kim et al., also validated the FT pumpless SOC model by evaluating antiaging product of curcuma longa leaf extract (CLLE). The results showed recovery of skin at 50 μg/mL dosage optimal for prevention of skin ageing [139]. The most appealing applications of SOC platforms are their potential to substitute the need of franz diffusion cell systems in pre-clinical applications, their utility in pre-clinical testing, investigating the development, and efficacy and toxicity of different pharmaceutical compounds like cosmetics, ointments, creams, antibiotics, and medicines.

Table 22.2 states the list of validation studies and techniques that have been used to develop various microfluidic SOC models, till date.

22.3.7 Disease-Specific Skin Biomarkers as End Points of Skin-on-Chip (SOC) Models

In vitro skin cultures are gaining importance in drug trials, mainly due to their ability to replicate the 3D microenvironment of the human skin. Identification of biomarkers is important to characterize healthy and diseased 3D models. Biomarkers can help in understanding the pathogenesis of diseases and various factors (genetic, environmental, etc.) that contribute to disease progression. Various skin disorders, such as psoriasis, photoageing, vitiligo, and cancers, like squamous cell carcinoma and melanoma, have been recapitulated as organotypic models and the disease markers have been measured and compared with control markers to identify the most prominent variations in their expression. Tables 22.3 and 22.4 present a list of all such healthy skin and diseased skin-related biomarkers that have been used as endpoints in skin diseases-on-chip models.

22.4 Application and Implementation of SOC Devices

22.4.1 Drug Testing and Toxicological Studies

Drug testing, an important stage of drug development, is the backbone of pharmaceutical industry. The overall process of drug development is as follows—(1) discovery and development of the drug in the laboratory; (2) pre-clinical research where the toxicity of the drugs is assessed in cellular systems; (3) The clinical research where the overall mechanism of action, beneficial effects, dosage on the organism, or physiological system is evaluated; (4) FDA approval of the drug by the advisory committee; and (5) post-market drug safety monitoring, with active surveillance

[211]. Among 10,000 new chemical entities, only a few are approved for transition into the market.

After target identification of a disease condition, the next steps include validation of the target through experimental data. This involves checking the effects on physiology, pathology, and molecular inhibition dynamics [212], which demands a robust system to recreate the in vivo environment under in vitro conditions. A novel, temperature controlled microfluidic SOC was developed by Lukács et al. for evaluation of drugs under realistic conditions [172]. The group evaluated penetration of caffeine as a cream (a hydrophilic model drug), in native and sensitized rat and mouse skins that were maintained within SOCs. This work was extended by Bajza et al. by studying irritation, corrosion potential, and efficiency of transdermal drug absorption through topical formulations. The effect of two P-glycoprotein substrate model drugs, e.g. quinidine and erythromycin, were studied in transdermal microdialysis in rat skin and in SOC devices. The transdermal absorption of drugs decreased after using inhibitors, and both freezing and ageing led to increased permeability of the skin. These findings make the SOC model ideal for investigating dermal barrier, skin penetration, and transport functionality of the skin [173].

Recent advancements have focussed on tissue-tissue and organ-organ interaction to better understand the effects or side effects of drugs on other organs. These multi-organ systems are connected by microfluidic channels, where metabolites can travel through the medium [213–215]. Wagner et al. established integrated human skin biopsies and human liver cells [146], where they observed longer cell viability. The model was exposed to various concentrations of the toxic agent, troglitazone, over a period of 6 days. This helped in understanding the effects of formulations like creams or cosmetics on the liver, when applied over skin or the effects of liver metabolites on the skin. An advanced model with integrated blood perfusion was established later [145]. Here, endothelialization was achieved by seeding dermal microvascular endothelial cells. mRNA analysis and immunohistochemistry exhibited liver sensitivity to troglitazone. Yet another research group has evaluated the toxic effects of doxorubicin on skin cells using a pumpless microfluidic human skin equivalent on-chip device, which was demonstrated as an effective drug testing platform [20]. Though multi-organ-on-chips are still at a nascent stage due to difficulties in recreating the complexity of each organ in vitro, SOCs are still better than the traditional cell culture methods for pre-clinical investigations of pharmaceuticals.

22.4.2 Wound Healing

Skin is the first barrier of the body that protects the tissues from microbial, physical, and chemical agents. In case of injury, the wounded skin repairs itself through a process involving the combination of complex cell behaviours. During wound healing, angiogenesis plays a vital role in supplying nutrients and oxygen to the healing tissues and removing the cellular waste. Hence, to mimic and test therapeutics on the healing tissue, vascularized SOC devices are useful. Along with the

influence of microenvironment and physiological cues, the involvement of cell proliferation, inflammation, and homeostasis plays a crucial role in wound healing. Biglari and co-workers created a wound-on-chip model to study the dermal wound healing process [216], wherein they mimicked the paracrine component of the early inflammation process that was similar to the wound healing phenomenon in terms of behaviour of various cell types. Human umbilical vein endothelial cells (HUVECs) and dermal fibroblasts were cultured in a wound-on-chip model with three interconnected channels. Tri-culturing with macrophages or addition of the tumour necrosis factor (TNF-α) resulted in a significant increase in interleukins (IL-8, IL-6 and IL-1β) and cytokine levels. This model exhibited potential to study the early inflammatory stages of wound healing and screening of anti-inflammatory drugs.

22.4.3 Diagnostics

The electronics and medical industries are progressing faster than ever before. The innovation of microfluidic, electronic devices has simplified disease diagnosis. Wearable devices and in vitro SOC devices are used to predict not only skin diseases, but also the state of illness in other organs or genetic disorders. These wearable devices focus on the sweat of the skin. Recently, Ray et al. showed how soft, skin-interfaced sweat stickers could successfully diagnose cystic fibrosis (CF), a genetic condition associated with the production of excess and thick mucus, which may lead to blockages and infection in the affected organs such as pancreas and lungs [217]. The concentration of chloride in the sweat acts as a robust biomarker for CF. Researchers introduced a sweat sticker, a soft epidermal microfluidic device that supported nearly perfect diagnosis via colourimetric validation, with the help of a smartphone camera. Another non-invasive wearable device is being developed by Epicore Biosystems, where researchers have designed a device with microchannels to measure inflammatory biomarkers, such as cytokines in sweat and intestinal fluids associated with atopic dermatitis, a highly pruritic, chronic inflammatory skin disease [218]. This further expands the field of colorimetric and digital assessment of parameters related to sweat rate, for sports science and measuring cortisol [219], glucose, vitamin C, and stress levels [220]. A full-thickness SOC model can be useful for identification of novel proteins or nucleic acid biomarkers [221] in diseased or skin cancer samples obtained from patients [222]. Researchers at the University of Illinois are working on a SOC model for a better understanding of the effects of ultraviolet rays (UV rays) on the skin. The device applies new chemistries to result in coloured changes depending upon the exposure dose of UV-A and UV-B. Combinatorially, the current advancements demonstrate a strong hope for the usefulness of SOC devices in diagnostics [223].

22.4.4 Inflammation

Inflammation is when the immune system in a human body responds to the infections, allergic components, internal disease conditions, physical forces, and external chemicals. This often results in formation of rashes on the skin, which may itch or burn, and may be flat or raised, smooth or scaly. Further, the inflamed area becomes warm and red. Exposure to UV, tissue damage, foreign bodies, or pathogen-induced cytokine cascade activates the defence system in the body [224–226]. Using SOCs has enabled investigations into understanding the effects of cosmetics and topical drugs on skin irritation and inflammation, which was earlier conducted in animals. Topical irritation was studied by Tavares et al. by applying fucoxanthin on a reconstructed human skin culture model containing both keratinocytes (KCs) and fibroblasts (Fbs). Fucoxanthin exhibited antioxidant functions and changes in interleukin factors, such as IL1α, IL-6, IL-8 were evaluated as inflammation markers, HSPB1 and EGFR as homeostasis markers, and NAT1 as metabolism marker [224]. The SOC model proposed by Wufuer et al. also supported simulation of inflammation within SOC devices. The cytokine expression level was evaluated upon induction with TNFα. Recent studies by Kwak et al. demonstrated the migratory immune responses by the leukocytes at the site of inflammation [134]. They co-cultured keratinocytes and dermal fibroblasts with vascular endothelial cells and verified the formation of vascular endothelium, as vasculature plays a major role in leukocyte migration to the sites of inflammation. Upon UV exposure, neutrophil migration was observed in the UV exposed area, along with an increased secretion of cytokines. These studies strengthen the future of SOC devices as a model for skin inflammation studies.

22.4.5 Skin Ageing

An aged skin often shows properties such as rough texture, wrinkling, loss of elasticity, laxity, etc. The ageing process has been studied in several SOC models. Kim et al. proved the anti-ageing effect of *Curcuma longa* leaf extract (a natural cosmetic ingredient) and co-enzyme Q10 via a pumpless chip, perfused with a gravity-driven flow [227, 228]. Fibroblasts and keratinocytes were co-cultured in a PDMS chip with two media reservoirs on both sides and a cylindrical chamber of 8 mm in the middle of the chip. A porous membrane was present in between two cell culture substituents. An enhanced barrier function and anti-ageing effects of the aforementioned compounds were observed. Pauty et al. studied the senescence in primary skin fibroblasts, in an elementary tissue-on-chip model, after exposing the cells to gamma-ray. 3D micro-vessels surrounded by young fibroblasts were imitated in a PDMS device where their ageing properties were evaluated with β-galactosidase assay [229]. The model co-facilitated successful observation of senescent-fibroblast-induced morphological changes in blood vessel, anti-ageing effects, and the study of senescence-associated secretory phenotype (SASP), in combination with SASP inhibitors useful for cancer research [229]. Skin wrinkling is another physical

phenomenon in which cells face different mechanical cues than normal conditions. Lim et al. developed wrinkled SOC, where they cultured keratinocytes and fibroblasts to form a 3D skin equivalent. A 10% stretch was introduced in this model for 12 h/day for 7 days, at the uniaxial frequency of 0.01 Hz or 0.05 Hz (generated by the attractive and repulsive force of the electromagnets) [141]. In both cases, the collagen, fibronectin, and keratin secretion decreased compared to non-stretched equivalents, indicating that the dermis suffered from repetitive tensile stress while stretching. This model was particularly effective for performing anti-ageing studies and for investigating anti-wrinkle cosmetics.

22.4.6 Studying Shear Stress

Shear stress is the tangential force per unit area acting on a surface. For example, it is the frictional force of the reagent or chemical or fluid tangentially applied on the tissue due to the flow. Shear stress has played a significant role in determining cell viability, in the field of microfluidics. It not only impacts the alignment or arrangement, but also affects growth, inflammation [230], permeability [231], porosity [232], and population of skin cells in a microfluidic chip. Lin et al. demonstrated the effects of higher shear stress on wound healing capabilities of the skin layer. Wounds were created in cultured embryonic fibroblast cells, NIH 3T3, by application of trypsin. Further, the rate of healing was analysed with β-lapachone, a wound healing promoter, at varying shear stress. It was observed that higher the shear stress, higher was the healing rate (total area healed/unit time) for larger wounds, at the concentration of 0.5 μM of β-lapachone. However, under shear stress of 0.174 mPa, the healing rate became linearly independent of β-lapachone concentration [231]. Suwannaphan et al. investigated how shear stress affected cell survival in a microfluidic chip, where the setup included needle, syringe, silicone tube, straight and spiral microchannel, and outlets. Their work involved computational simulation and was validated by using devices for observing cell survival, under microscope, with a vital dye. In this work, the survival of the cultured cells was optimized by controlling the flow rate (volume of fluid/unit time) [233]. Agarwal et al. studied the effect of shear stress during embryogenesis, at both individual keratinocyte cells and collective cell layers. During embryogenesis, the developing skin spends a certain duration in amniotic fluid and responds to shear stress when fluid flows over it. A low shear stress of 0.06 dyne/cm^2 was observed to induce improved epithelium formation by cellular cytoskeletal and morphological reorganization. On the other hand, higher shear of 6 dyne/cm^2 resulted in cellular disruption. Also, increase in nuclear lamin expression is related to shear stress, which suggests that mechanical signals transmit to the nucleus, making this model well suited for understanding organogenesis of the epidermis [234].

22.5 Impact, IPR, and Regulations of SOC Technology

22.5.1 Social Impact

The skin-on-a-chip models offer social benefits like improved drug testing methods and reduced use of animal models. These physiologically and pathologically relevant in vitro models offer multiple benefits in clinical research like faster identification of potential drug candidates, personalized medicine by testing drugs on organ-on-chips fabricated using patients' own cells, development of effective treatments with minimum failure rate, development of efficient disease models to study drug mechanisms, etc. Due to fewer drug assessment failures and reduction in animal experiments, it would be possible to provide a better quality of healthcare to the society, at a lower cost.

22.5.2 Industrial Impact

Skin-on-a-chips (SOC) have a significant impact on the pharmaceutical industry. Other industries like the food industry (for testing allergens in food products), the cosmetic industry (for testing cosmetic products on skin for potential harmful effects), and the chemical industry (for testing dangerous effects of chemicals) have also shown interest in these models. These new segments will allow the development of different organ-on-chip models and efficient drug discovery techniques [235].

22.5.3 Economic Impact

R&D involves high costs for pharmaceutical biotechnology companies to enter the market, which limits innovations and leads to high costs of drugs. The success at the R&D level is determined by success rates, duration of development, and direct expenses per project [236]. Insufficient efficacy and safety are the major concerns related to drugs, which lead to the failure of projects, but which is also the driver of costs [237, 238]. Skin-on-chip models, which mimic the human physiology very closely, will play an important role in bridging the gap between human trials and pre-clinical testing and impact the high R&D costs [239, 240]. Experts have confirmed about 10–26% cost reduction in R&D costs due to this emerging technology, which has been projected to save around US $169 million per new drug launched in the market [241–243]. Fewer projects based on skin-on-chip, which are predictive in early phases and give precise results, are needed to launch new drugs at lower R&D costs. The exact economic impact due to this evolving technology needs to be explored further and also social discussions in terms of lower drug prices need to be considered.

22.5.4 Regulations and IPR

The FDA's office of Clinical Pharmacology, the Centre of Drug Evaluation and Research (CDER), and the Division of Applied Regulatory Science reported organ-on-chip models as new emerging technologies for disease assessment and for testing drug's efficacy and safety [244]. Clinical Laboratory Improvement Amendments (CLIA) regulatory standards for diagnostic facilities and disease treatments, given by U.S. Centres of Medicare and Medicaid Service, may apply to organ on chip technology. It includes audits on quality management, reliability of the data, specimen integrity, quality testing as well as techniques of calibrations and correction [235].

For pre-clinical toxicological studies, the U.S. FDA has established regulatory guidelines for Good Laboratory Practice for Nonclinical Laboratory Studies (21CFR Part 58), which includes testing facility management, requirements for specimen storage, standard operating procedures, keeping track of expiry dates of chemicals and reagents, equipment cleaning and calibration as well as archiving of reports and data [245]. 3D human reconstructed epidermis models have been validated for skin corrosivity, irritation, sensitization, phototoxicity, and genotoxicity testing under Organisation for Economic Co-operation and Development (OECD) guidelines 431, 439, 498 [246–248].

Private companies own intellectual property rights for organs on chips. Organs-on-chips have applications in the field of drug discovery and development and are protected under different intellectual property rights. The codes and softwares used to fabricate these devices are protected through copyright. Intellectual property protects the use of various polymers through patents [235]. These frameworks of regulatory standards help to produce reliable and reproducible data through organ-on-chip models.

22.6 Challenges in SOC Development and Future Perspective

The main challenge with these technologies is the production of constructs having larger dimensions, which consequently increases the overall cost of production. Also, many of the models are not amenable to high-throughput pre-clinical and translational studies. Advanced research has introduced methodologies for constructing epidermal-dermal tissue interface in a microfluidic set up by using syringe pumps, thus overcoming the manual alterations during cell culture. However, these investigations are preliminary and efficient differentiation of epidermal layers has neither been achieved nor characterized.

Troubles associated with development of fully functional skin-on-chip are numerous. Several studies have highlighted the problem of matrix contraction and degradation mediated by skin fibroblasts, which resulted in decreased reproducibility and limited lifespan of the on-chip models. Researchers have also reported poor physiochemical properties like acute shrinkage, loose attachment of skin reconstructs in the culture inserts/membrane, and poor biomechanical properties. Furthermore,

development of microfluidic SOC devices needs experienced and trained manpower, with a creative and scientific acumen, to create and implement protocols and facilitate the desired output.

22.6.1 Limitations in SOC Co-culture Architecture

Co-culture SOC designs can be categorized as vertical and planar, depending on the orientation of the microfluidic culture chambers. Each design comes with its own advantages and disadvantages. SOCs having multi-layered, vertical co-culture designs are composed of stacked cell culture chambers having porous membranes in between. This allows cells to be confined within the chamber, while allowing biomolecules or biochemicals to flow freely through the porous membranes [249]. The major limitations of this design are that the imaging of cells is problematic due to difficulties in imaging through the membrane, which complicates the imaging of both cell types. In contrast, the planar co-culture design is ideal for imaging and fluid control as the layers lie in the same plane. Additional challenges are presented by the multilayer designs, which makes microfabrication more demanding, especially while mimicking the organs with three or more layers. This, in turn, limits the applicability of these designs. Planar co-cultures contain parallel cell culture chambers separated by microchannel arrays or porous gels [250–252]. This design is unable to imitate the tight junction barrier of the skin and the distance between the co-culture chambers is greater, as compared to the vertical design. Thus, no SOC design fulfils all the criteria for recreating the in vivo architecture. Each SOC design displays its own disadvantages or limitations. Hence, understanding the 'fit for purpose' concept becomes critical while designing the devices.

22.6.2 Limitations of PDMS-Based SOCs

PDMS is one of the most used materials in the field of soft lithography. It shows excellent biocompatibility along with appropriate optical, chemical, and mechanical properties, such as permeability and transparency. But it suffers from various issues due to its high rigidity and transient nature of surface modifications that limit the long-term cell culture in PDMS devices. The major problem arises due to the non-specific absorption of less hydrophobic molecules, drugs [253], and proteins by PDMS itself. This complicates accurate analysis of drugs [254–256].

Alternatives to PDMS may overcome these problems and help in mimicking organ microenvironments within SOCs. Alginate is mechanically tunable and chemically functionalized for cell attachment and thus, better mimics the soft tissue microenvironment and maintains cell viability [257]. Materials such as polytetrafluoroethylene (PTFE), polymethyl methacrylate (PMMA), perfluoropolyether (PFPE), polyimides, styrene-ethylene-butylene-styrene (SEBS),

and thermostat polyesters are some of the considerable alternative materials for PDMS [258, 259].

22.6.3 Limitation in Throughput of Cell Injection, Perfusion, and Sampling

The throughput of SOCs needs to be scaled up with precisely controlled media, samples, and target compound injection, in an automated manner, to enable parallel experimentation. Thus, integration of automation hardware, liquid handling systems, or low-cost valve/pumping **systems** within the existing devices is required [260]. Injection of cells into the inlet ports devices are still performed by manual pipetting. Automation will enable sterile handling of cells and will allow rapid and precise injections, by avoiding any cellular stress. Though some HT-SOCs have recently been investigated, many of these approaches lack amenability to high-throughput screening which necessitates significant time for analysis of a large number of samples.

22.6.4 Lack of Integrated Online Analytics of Biosensors

Although biosensors have been integrated in a very few OOC devices for sensing biological phenomena or secretory molecules, the capacity to monitor physiological changes in many samples, in response to chemical or physical cues, is still far from reality. The current OOC devices feature electrical [261], electrochemical [22], and optical chemical [262] sensors, which are limited in terms of reproducibility, sensitivity, and measuring range. The optical sensors allow online measurement of pH, temperature, and O_2 concentrations [263]. However, electrochemical sensors allow measurement of glucose, lactate levels, antigen levels, etc. [264]. In a study done by Ramadan and Sriram, electrodes were incorporated for measuring real-time TEER values resulting from proliferation and differentiation of skin [21]. Using multiple sensors will enhance data collection and enable multiple readouts, parallelly. An integrated computerized data collection module will enhance the efficacy of the device, with least manual involvement. Although the biological as well technical challenges of microfluidic SOCs require further mitigation, these devices have immense potential to revolutionize the pre-clinical research, through development of normal and diseased skin models.

22.7 Current Status and Future Perspectives on Skin-on-a-Chip (SOC) Technology

Models mimicking native skin tissue play a significant role in evaluating the response of skin to various factors like pathogens, drugs, cosmetics, UV radiations, in developing skin transplants, in understanding disease mechanisms, and in

screening novel drugs for treating skin diseases. There are various commercially available skin models used for these purposes and complex SOC models are yet to be developed. Reconstructed human epidermis models include RHE™, EpiSkin™, EpiDerm™ and StratiCELL, which are fabricated using keratinocytes and have been proven as beneficial for pigmentation studies, permeability studies, to evaluate mechanisms of adhesive bacteria, etc. [67]. The commercially available reconstructed skin models include Phenion®, TSkin™, StrataTest®, and EpiDermFT™, which are composed of an epidermis (keratinocytes) and a dermal layer (fibroblast cells). The Melanoma™ model has been developed including human malignant melanoma cells, to study cancerous conditions [265].

Skin grafts and freeze-dried scaffolds are fabricated using biopolymers to replace and restore the barrier function of injured/burnt skin. Various such models are being developed like Alloderm® from human cadaveric skin and Integra® consisting of collagen and glycosaminoglycan matrix [266, 267]. Models such as Apligraf®, Dermagraft ™, and Transcyte® [268, 269] have been developed for healing of skin wounds/ulcers. Epicel® is an autograft fabricated using patient's own skin cells [270]. Stratagraft® is a full-thickness skin model developed using dermal fibroblasts and normal immortalized keratinocytes, which is under clinical trial [271]. A genetically engineered skin model was recently developed as a treatment for diabetic foot ulcers. These models, ExpressedGraft™-C9T1 and ExpressGraft™, that express human vascular endothelial growth factor (VEGFs) are currently being evaluated in clinical trials [272].

All the above-mentioned models can be used for various studies. Yet these models lack proper vascularization, variety of cells, and mechanical stability, due to which they cannot efficiently mimic the functionality of the native skin. Also, other factors affecting the model design are difficulties faced while growing cells with polymers, scaffolds lacking mechanical resistance, etc. SOC models possess the capacity to overcome these limitations as they mimic skin microenvironment and vascularization and also allow cell-cell/cell-matrix interactions. Introduction of ECM proteins, immune cells, skin appendages, and genetically modified cells is feasible in such models and hence they are emerging as new and beneficial opportunities in the field of tissue engineering [52, 89, 93].

The development of fully functional skin models requires collaboration between scientists from diverse fields. The SOC platforms should be affordable and easy to operate. Alternatives to hydrogels/polymers should be discovered to accurately mimic ECM. Blood vessels with the dimensions of capillaries should be developed to maintain proper interactions between endothelial cells and fibroblast cells and to maintain proper blood pressure for the diffusion of biomolecules. Facilities like real-time monitoring and automated analysis of the health of the tissue using biosensors require further development. Patient-specific testing can be conducted by developing SoC models using iPSCs derived from patients.

In the future, the SOC models will be available for advanced applications and research practices, which will aid and expand our understanding of skin and skin diseases, to provide standardized, novel treatments. These models will be

advantageous in drug/cosmetic screening, disease modelling, and in the development of personalized medicines.

References

1. Abaci HE, Guo Z, Doucet Y, Jacków J, Christiano A (2017) Next generation human skin constructs as advanced tools for drug development. Exp Biol Med (Maywood) 242(17): 1657–1668. https://doi.org/10.1177/1535370217712690
2. Effects of air pollution on the skin: a review. Indian J Dermatol Venereol Leprol. June 30, 2017. https://ijdvl.com/effects-of-air-pollution-on-the-skin-a-review/. Accessed 31 Oct 2021
3. Svobodová A, Vostálová J (2010) Solar radiation induced skin damage: review of protective and preventive options. Int J Radiat Biol 86(12):999–1030. https://doi.org/10.3109/09553002.2010.501842
4. Karimkhani C et al (2017) Global skin disease morbidity and mortality. JAMA Dermatol 153(5):406–412. https://doi.org/10.1001/jamadermatol.2016.5538
5. Karimkhani C et al (2017) Global skin disease morbidity and mortality: an update from the global burden of disease study 2013. JAMA Dermatol 153(5):406–412. https://doi.org/10.1001/jamadermatol.2016.5538
6. Prausnitz MR, Langer R (2008) Transdermal drug delivery. Nat Biotechnol 26(11):11. https://doi.org/10.1038/nbt.1504
7. Eungdamrong NJ et al (2014) Challenges and promises in modeling dermatologic disorders with bioengineered skin. Exp Biol Med (Maywood) 239(9):1215–1224. https://doi.org/10.1177/1535370214538747
8. Yun Y, Jung YJ, Choi Y, Choi JS, Cho YW (2018) Artificial skin models for animal-free testing. J Pharm Investig 48. https://doi.org/10.1007/s40005-018-0389-1
9. Zhang Q, Sito L, Mao M, He J, Zhang YS, Zhao X (2018) Current advances in skin-on-a-chip models for drug testing. Microphysiol Syst 2. https://doi.org/10.21037/mps.2018.08.01
10. Esch MB, King TL, Shuler ML (2011) The role of body-on-a-chip devices in drug and toxicity studies. Annu Rev Biomed Eng 13(1):55–72. https://doi.org/10.1146/annurev-bioeng-071910-124629
11. Esch EW, Bahinski A, Huh D (2015) Organs-on-chips at the frontiers of drug discovery. Nat Rev Drug Discov 14(4):248–260. https://doi.org/10.1038/nrd4539
12. Ferdowsian HR, Beck N (2011) Ethical and scientific considerations regarding animal testing and research. PLoS One. https://doi.org/10.1371/journal.pone.0024059
13. Von Aulock S (2019) Is there an end in sight for animal testing? Can organ-on-a-chip replace animal use in safety testing with advanced human-focused approaches? ALTEX. https://doi.org/10.14573/altex.1812121
14. Lilienblum W et al (2008) Alternative methods to safety studies in experimental animals: role in the risk assessment of chemicals under the new European Chemicals Legislation (REACH). Arch Toxicol. https://doi.org/10.1007/s00204-008-0279-9
15. Almeida A, Sarmento B, Rodrigues F (2017) Insights on in vitro models for safety and toxicity assessment of cosmetic ingredients. Int J Pharm. https://doi.org/10.1016/j.ijpharm.2017.01.024
16. Hewitt NJ et al (2013) Use of human in vitro skin models for accurate and ethical risk assessment: metabolic considerations. Toxicol Sci. https://doi.org/10.1093/toxsci/kft080
17. Astashkina A, Grainger DW (2014) Critical analysis of 3-D organoid in vitro cell culture models for high-throughput drug candidate toxicity assessments. Adv Drug Deliv Rev. https://doi.org/10.1016/j.addr.2014.02.008
18. Bhatia SN, Ingber DE (2014) Microfluidic organs-on-chips. Nat Biotechnol 32(8):760–772. https://doi.org/10.1038/nbt.2989

19. Mohammadi MH et al (2016) Skin diseases modeling using combined tissue engineering and microfluidic technologies. Adv Healthc Mater 5(19):2459–2480. https://doi.org/10.1002/adhm.201600439
20. Abaci HE, Gledhill K, Guo Z, Christiano AM, Shuler ML (2015) Pumpless microfluidic platform for drug testing on human skin equivalents. Lab Chip 15(3):882–888. https://doi.org/10.1039/c4lc00999a
21. Sriram G et al (2018) Full-thickness human skin-on-chip with enhanced epidermal morphogenesis and barrier function. Mater Today 21(4):326–340. https://doi.org/10.1016/j.mattod.2017.11.002
22. Zhang YS et al (2017) Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1612906114
23. Kilic T, Navaee F, Stradolini F, Renaud P, Carrara S (2018) Organs-on-chip monitoring: sensors and other strategies. Microphysiol Syst. https://doi.org/10.21037/mps.2018.01.01
24. Seok J et al (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1222878110
25. Mak IWY, Evaniew N, Ghert M (2014) Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res 6:114–118
26. Wufuer M et al (2016) Skin-on-a-chip model simulating inflammation, edema and drug-based treatment. Sci Rep 6(August):1–12. https://doi.org/10.1038/srep37471
27. Ramadan Q, Ting FCW (2016) In vitro micro-physiological immune-competent model of the human skin. Lab Chip. https://doi.org/10.1039/c6lc00229c
28. Ghaemmaghami AM, Hancock MJ, Harrington H, Kaji H, Khademhosseini A (2012) Biomimetic tissues on a chip for drug discovery. Drug Discov Today 17(3–4):173–181. https://doi.org/10.1016/j.drudis.2011.10.029
29. Pati F, Gantelius J, Svahn HA (2016) 3D bioprinting of tissue/organ models. Angew Chem Int Ed 55(15):4650–4665. https://doi.org/10.1002/anie.201505062
30. Au AK, Lee W, Folch A (2014) Mail-order microfluidics: evaluation of stereolithography for the production of microfluidic devices. Lab Chip 14(7):1294–1301. https://doi.org/10.1039/C3LC51360B
31. Selimović S, Dokmeci MR, Khademhosseini A (2013) Organs-on-a-chip for drug discovery. Curr Opin Pharmacol 13(5):829–833. https://doi.org/10.1016/j.coph.2013.06.005
32. Zheng F, Fu F, Cheng Y, Wang C, Zhao Y, Gu Z (2016) Organ-on-a-chip systems: microengineering to biomimic living systems. Small 12(17):2253–2282. https://doi.org/10.1002/smll.201503208
33. Organs-on-chip monitoring: sensors and other strategies - Kilic - Microphysiological Systems. https://mps.amegroups.com/article/view/4689/5479. Accessed 20 June 2021
34. Schulz JT III, Tompkins RG, Burke JF (2000) Artificial skin. Annu Rev Med 51(1):231–244. https://doi.org/10.1146/annurev.med.51.1.231
35. Sorrell JM, Caterson B, Caplan AI, Davis B, Schafer IA (1990) Human keratinocytes contain carbohydrates that are recognized by keratan sulfate-specific monoclonal antibodies. J Invest Dermatol 95(3):347–352. https://doi.org/10.1111/1523-1747.ep12485110
36. Vijayavenkataraman S, Lu WF, Fuh JYH (2016) 3D bioprinting of skin: a state-of-the-art review on modelling, materials, and processes. Biofabrication 8(3):032001. https://doi.org/10.1088/1758-5090/8/3/032001
37. Elsevier Enhanced Reader. https://doi.org/10.1016/j.bbamem.2006.06.021. https://reader.elsevier.com/reader/sd/pii/S0005273606002410?token=C6DA4EC4BF5B7A11B86ACF3128CA110E6258177FD1ADC7578F27243DEFF07B0FC40E0EF30B9F2266E3B8AE9CB8C9BF4A&originRegion=eu-west-1&originCreation=20210722094219. Accessed 22 July 2021
38. Lin JY, Fisher DE (2007) Melanocyte biology and skin pigmentation. Nature 445(7130):843–850. https://doi.org/10.1038/nature05660

39. Choi H-I et al (2014) Melanosome uptake is associated with the proliferation and differentiation of keratinocytes. Arch Dermatol Res 306(1):59–66. https://doi.org/10.1007/s00403-013-1422-x
40. Doebel T, Voisin B, Nagao K (2017) Langerhans cells – the macrophage in dendritic cell clothing. Trends Immunol 38(11):817–828. https://doi.org/10.1016/j.it.2017.06.008
41. Lumpkin EA, Caterina MJ (2007) Mechanisms of sensory transduction in the skin. Nature 445(7130):858–865. https://doi.org/10.1038/nature05662
42. Woo S-H, Lumpkin EA, Patapoutian A (2015) Merkel cells and neurons keep in touch. Trends Cell Biol 25(2):74–81. https://doi.org/10.1016/j.tcb.2014.10.003
43. Pereira RF, Barrias CC, Granja PL, Bartolo PJ (2013) Advanced biofabrication strategies for skin regeneration and repair. Nanomedicine (Lond) 8(4):603–621. https://doi.org/10.2217/nnm.13.50
44. Dias J, Granja P, Bártolo P (2016) Advances in electrospun skin substitutes. Prog Mater Sci 84:314–334. https://doi.org/10.1016/j.pmatsci.2016.09.006
45. Planz V, Lehr C-M, Windbergs M (2016) In vitro models for evaluating safety and efficacy of novel technologies for skin drug delivery. J Control Release 242:89–104. https://doi.org/10.1016/j.jconrel.2016.09.002
46. Kahan V, Andersen ML, Tomimori J, Tufik S (2009) Stress, immunity and skin collagen integrity: evidence from animal models and clinical conditions. Brain Behav Immun 23(8):1089–1095. https://doi.org/10.1016/j.bbi.2009.06.002
47. Casey G (2002) Physiology of the skin. Nurs Stand 16(34):47–51.; quiz 53, 55. https://doi.org/10.7748/ns2002.05.16.34.47.c3193
48. Papakonstantinou E, Roth M, Karakiulakis G (2012) Hyaluronic acid: a key molecule in skin aging. Dermatoendocrinology 4(3):253–258. https://doi.org/10.4161/derm.21923
49. Freundlich B, Bomalaski JS, Neilson E, Jimenez SA (1986) Regulation of fibroblast proliferation and collagen synthesis by cytokines. Immunol Today 7(10):303–307. https://doi.org/10.1016/0167-5699(86)90067-8
50. Krishnaswamy G, Ajitawi O, Chi DS (2006) The human mast cell: an overview. Methods Mol Biol 315:13–34. https://doi.org/10.1385/1-59259-967-2:013
51. Lai-Cheong JE, McGrath JA (2017) Structure and function of skin, hair and nails. Medicine 45(6):347–351. https://doi.org/10.1016/j.mpmed.2017.03.004
52. Lee S, Jin SP, Kim YK, Sung GY, Chung JH, Sung JH (2017) Construction of 3D multicellular microfluidic chip for an in vitro skin model. Biomed Microdevices. https://doi.org/10.1007/s10544-017-0156-5
53. Nishimura SI, Ueda M, Sasai M (2009) Cortical factor feedback model for cellular locomotion and cytofission. PLoS Comput Biol. https://doi.org/10.1371/journal.pcbi.1000310
54. Liang CC, Park AY, Guan JL (2007) In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. Nat Protoc. https://doi.org/10.1038/nprot.2007.30
55. Vedel S, Tay S, Johnston DM, Bruus H, Quake SR (2013) Migration of cells in a social context. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.1204291110
56. Polini A, Prodanov L, Bhise NS, Manoharan V, Dokmeci MR, Khademhosseini A (2014) Organs-on-a-chip: a new tool for drug discovery. Expert Opin Drug Discovery 9(4):335–352. https://doi.org/10.1517/17460441.2014.886562
57. van den Broek LJ, Bergers LIJC, Reijnders CMA, Gibbs S (2017) Progress and future prospectives in skin-on-chip development with emphasis on the use of different cell types and technical challenges. Stem Cell Rev Rep 13(3):418–429. https://doi.org/10.1007/s12015-017-9737-1
58. Duval K et al (2017) Modeling physiological events in 2D vs. 3D Cell Culture. Physiology (Bethesda) 32(4):266–277. https://doi.org/10.1152/physiol.00036.2016
59. Hinz B, Gabbiani G (2003) Cell-matrix and cell-cell contacts of myofibroblasts: role in connective tissue remodeling. Thromb Haemost 90(12):993–1002. https://doi.org/10.1160/TH03-05-0328

60. Bhatia SN, Ingber DE (2014) Microfluidic organs-on-chips. Nat Biotechnol 32(8):8. https://doi.org/10.1038/nbt.2989
61. Parmaksiz M, Dogan A, Odabas S, Elçin AE, Elçin YM (2016) Clinical applications of decellularized extracellular matrices for tissue engineering and regenerative medicine. Biomed Mater 11(2):022003. https://doi.org/10.1088/1748-6041/11/2/022003
62. Smola H, Thiekötter G, Fusenig NE (1993) Mutual induction of growth factor gene expression by epidermal-dermal cell interaction. J Cell Biol 122(2):417–429. https://doi.org/10.1083/jcb.122.2.417
63. Schmidt BA, Horsley V (2013) Intradermal adipocytes mediate fibroblast recruitment during skin wound healing. Development 140(7):1517–1527. https://doi.org/10.1242/dev.087593
64. Nie J et al (2018) Vessel-on-a-chip with hydrogel-based microfluidics. Small 14(45): e1802368. https://doi.org/10.1002/smll.201802368
65. Kim BS, Lee J-S, Gao G, Cho D-W (2017) Direct 3D cell-printing of human skin with functional transwell system. Biofabrication 9(2):025034. https://doi.org/10.1088/1758-5090/aa71c8
66. Sano E et al (2019) Tetrafluoroethylene-propylene elastomer for fabrication of microfluidic organs-on-chips resistant to drug absorption. Micromachines 10(11):11. https://doi.org/10.3390/mi10110793
67. Fentem JH, Botham PA (2002) ECVAM's activities in validating alternative tests for skin corrosion and irritation. https://journals.sagepub.com/doi/abs/10.1177/026119290203002S09. Accessed 08 July 2021
68. Pellevoisin C et al (2018) SkinEthic™ RHE for in vitro evaluation of skin irritation of medical device extracts. Toxicol In Vitro 50:418–425. https://doi.org/10.1016/j.tiv.2018.01.008
69. Asbill C, Kim N, El-Kattan A, Creek K, Wertz P, Michniak B (2000) Evaluation of a human bio-engineered skin equivalent for drug permeation studies. Pharm Res 17(9):1092–1097. https://doi.org/10.1023/a:1026405712870
70. Ouwehand K, Spiekstra SW, Waaijman T, Scheper RJ, de Gruijl TD, Gibbs S (2011) Technical advance: Langerhans cells derived from a human cell line in a full-thickness skin equivalent undergo allergen-induced maturation and migration. J Leukoc Biol 90(5): 1027–1033. https://doi.org/10.1189/jlb.0610374
71. Tremblay P-L, Berthod F, Germain L, Auger FA (2005) In vitro evaluation of the angiostatic potential of drugs using an endothelialized tissue-engineered connective tissue. J Pharmacol Exp Ther 315(2):510–516
72. Characterization of a human skin equivalent model to study the effects of ultraviolet B radiation on keratinocytes. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4074762/. Accessed 08 July 2021
73. Portes P, Pygmalion MJ, Popovic E, Cottin M, Mariani M (2002) Use of human reconstituted epidermis Episkin for assessment of weak phototoxic potential of chemical compounds. Photodermatol Photoimmunol Photomed 18(2):96–102. https://doi.org/10.1034/j.1600-0781.2002.180207.x
74. Dreher F, Fouchard F, Patouillet C, Andrian M, Simonnet J-T, Benech-Kieffer F (2002) Comparison of cutaneous bioavailability of cosmetic preparations containing caffeine or alpha-tocopherol applied on human skin models or human skin ex vivo at finite doses. Skin Pharmacol Appl Ski Physiol 15(Suppl 1):40–58. https://doi.org/10.1159/000066680
75. Dreher F et al (2002) Improvement of the experimental setup to assess cutaneous bioavailability on human skin models: dynamic protocol. Skin Pharmacol Appl Ski Physiol 15(Suppl 1): 31–39. https://doi.org/10.1159/000066683
76. Gibbs S, Vietsch H, Meier U, Ponec M (2002) Effect of skin barrier competence on SLS and water-induced IL-1alpha expression. Exp Dermatol 11(3):217–223. https://doi.org/10.1034/j.1600-0625.2001.110304.x
77. Schmook FP, Meingassner JG, Billich A (2001) Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. Int J Pharm 215(1): 51–56. https://doi.org/10.1016/S0378-5173(00)00665-7

78. Guth K, Schäfer-Korting M, Fabian E, Landsiedel R, van Ravenzwaay B (2015) Suitability of skin integrity tests for dermal absorption studies in vitro. Toxicol In Vitro 29(1):113–123. https://doi.org/10.1016/j.tiv.2014.09.007
79. Groeber F, Holeiter M, Hampel M, Hinderer S, Schenke-Layland K (2011) Skin tissue engineering--in vivo and in vitro applications. Adv Drug Deliv Rev 63(4–5):352–366. https://doi.org/10.1016/j.addr.2011.01.005
80. Werner S, Krieg T, Smola H (2007) Keratinocyte–fibroblast interactions in wound healing. J Investig Dermatol 127(5):998–1008. https://doi.org/10.1038/sj.jid.5700786
81. Schumacher M et al (2014) Efficient keratinocyte differentiation strictly depends on JNK-induced soluble factors in fibroblasts. J Invest Dermatol 134(5):1332–1341. https://doi.org/10.1038/jid.2013.535
82. Catarino CM et al (2018) Skin corrosion test: a comparison between reconstructed human epidermis and full thickness skin models. Eur J Pharm Biopharm 125:51–57. https://doi.org/10.1016/j.ejpb.2018.01.002
83. Thakoersing VS, Ponec M, Bouwstra JA (2010) Generation of human skin equivalents under submerged conditions—mimicking the in utero environment. Tissue Eng A 16(4):1433–1441. https://doi.org/10.1089/ten.tea.2009.0358
84. Mallampati R et al (2010) Evaluation of EpiDerm full thickness-300 (EFT-300) as an in vitro model for skin irritation: Studies on aliphatic hydrocarbons. Toxicol In Vitro 24(2):669–676. https://doi.org/10.1016/j.tiv.2009.08.019
85. Heisler E, Hoffmann JJ, Peters P, Ahr HJ, Vohr H-W (2003) 155 Advanced skin test 2000 (AST-2000) as a potent in vitro tool for the characterization of skin reactions by protein fingerprinting. Toxicol Lett 144:s44–s45. https://doi.org/10.1016/S0378-4274(03)90154-8
86. Rasmussen C et al (2010) The StrataTest® human skin model, a consistent in vitro alternative for toxicological testing. Toxicol In Vitro 24:2021–2029. https://doi.org/10.1016/j.tiv.2010.07.027
87. Kumar R, Parsad D, Kanwar A, Kaul D (2012) Development of melanocye-keratinocyte co-culture model for controls and vitiligo to assess regulators of pigmentation and melanocytes. Indian J Dermatol Venereol Leprol 78(5):599–604. https://doi.org/10.4103/0378-6323.100567
88. Kosten IJ, Spiekstra SW, de Gruijl TD, Gibbs S (2015) MUTZ-3 derived Langerhans cells in human skin equivalents show differential migration and phenotypic plasticity after allergen or irritant exposure. Toxicol Appl Pharmacol 287(1):35–42. https://doi.org/10.1016/j.taap.2015.05.017
89. Chau DYS, Johnson C, MacNeil S, Haycock JW, Ghaemmaghami AM (2013) The development of a 3D immunocompetent model of human skin. Biofabrication 5(3):035011. https://doi.org/10.1088/1758-5082/5/3/035011
90. van den Bogaard EH et al (2014) Crosstalk between keratinocytes and T cells in a 3D microenvironment: a model to study inflammatory skin diseases. J Investig Dermatol 134(3):719–727. https://doi.org/10.1038/jid.2013.417
91. Monfort A, Soriano-Navarro M, García-Verdugo JM, Izeta A (2013) Production of human tissue-engineered skin trilayer on a plasma-based hypodermis. J Tissue Eng Regen Med 7(6):479–490. https://doi.org/10.1002/term.548
92. Trottier V, Marceau-Fortier G, Germain L, Vincent C, Fradette J (2008) IFATS collection: using human adipose-derived stem/stromal cells for the production of new skin substitutes. Stem Cells 26(10):2713–2723. https://doi.org/10.1634/stemcells.2008-0031
93. Abaci HE et al (2016) Human skin constructs with spatially controlled vasculature using primary and iPSC-derived endothelial cells. Adv Healthc Mater 5(14):1800–1807. https://doi.org/10.1002/adhm.201500936
94. Wufuer M et al (2016) Skin-on-a-chip model simulating inflammation, edema and drug-based treatment. Sci Rep 6(1):37471. https://doi.org/10.1038/srep37471
95. Dhanwada KR, Garrett L, Smith P, Thompson KD, Doster A, Jones C (1993) Characterization of human keratinocytes transformed by high risk human papillomavirus types 16 or 18 and

herpes simplex virus type 2. J Gen Virol 74(Pt 6):955–963. https://doi.org/10.1099/0022-1317-74-6-955
96. Schoop VM, Mirancea N, Fusenig NE (1999) Epidermal organization and differentiation of HaCaT keratinocytes in organotypic coculture with human dermal fibroblasts. J Invest Dermatol 112(3):343–353. https://doi.org/10.1046/j.1523-1747.1999.00524.x
97. Reijnders CMA, van Lier A, Roffel S, Kramer D, Scheper RJ, Gibbs S (2015) Development of a full-thickness human skin equivalent in vitro model derived from TERT-immortalized keratinocytes and fibroblasts. Tissue Eng Part A 21(17–18):2448–2459. https://doi.org/10.1089/ten.TEA.2015.0139
98. Okugawa Y, Hirai Y (2008) Overexpression of extracellular epimorphin leads to impaired epidermal differentiation in HaCaT keratinocytes. J Invest Dermatol 128(8):1884–1893. https://doi.org/10.1038/jid.2008.22
99. Ataç B et al (2013) Skin and hair on-a-chip: in vitro skin models versus ex vivo tissue maintenance with dynamic perfusion. Lab Chip 13(18):3555. https://doi.org/10.1039/c3lc50227a
100. Mori N, Morimoto Y, Takeuchi S (2017) Skin integrated with perfusable vascular channels on a chip. Biomaterials 116:48–56. https://doi.org/10.1016/j.biomaterials.2016.11.031
101. Sandby-Møller J, Poulsen T, Wulf HC (2003) Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits. Acta Derm Venereol 83(6):410–413. https://doi.org/10.1080/00015550310015419
102. Hwa C, Bauer EA, Cohen DE (2011) Skin biology: systems that enhance drug delivery - skin biology. Dermatol Ther 24(5):464–470. https://doi.org/10.1111/j.1529-8019.2012.01460.x
103. Oltulu P, Ince B, Kokbudak N, Findik S, Kilinc F (2018) Measurement of epidermis, dermis, and total skin thicknesses from six different body regions with a new ethical histometric technique. Turk J Plast Surg 26(2):56. https://doi.org/10.4103/tjps.TJPS_2_17
104. Gangatirkar P, Paquet-Fifield S, Li A, Rossi R, Kaur P (2007) Establishment of 3D organotypic cultures using human neonatal epidermal cells. Nat Protoc 2(1):178–186. https://doi.org/10.1038/nprot.2006.448
105. Itoh M, Umegaki-Arao N, Guo Z, Liu L, Higgins CA, Christiano AM (2013) Generation of 3D skin equivalents fully reconstituted from human induced pluripotent stem cells (iPSCs). PLoS One 8(10):e77673. https://doi.org/10.1371/journal.pone.0077673
106. Liu Y, Ma L, Gao C (2012) Facile fabrication of the glutaraldehyde cross-linked collagen/chitosan porous scaffold for skin tissue engineering. Mater Sci Eng C 32(8):2361–2366. https://doi.org/10.1016/j.msec.2012.07.008
107. Sheikh F et al (2014) 3D electrospun silk fibroin nanofibers for fabrication of artificial skin. Nanomed Nanotechnol, Biol Med 11. https://doi.org/10.1016/j.nano.2014.11.007
108. Cheung HY, Lau KT, Lu TP, Hui D (2007) A critical review on polymer-based bio-engineered materials for scaffold development. Compos B Eng. https://doi.org/10.1016/j.compositesb.2006.06.014
109. Golas AR, Hernandez KA, Spector JA (2014) Tissue engineering for plastic surgeons: a primer. Aesth Plast Surg 38(1):207–221. https://doi.org/10.1007/s00266-013-0255-5
110. Pereira RF, Barrias CC, Granja PL, Bartolo PJ (2013) Advanced biofabrication strategies for skin regeneration and repair. Nanomedicine 8(4):603–621. https://doi.org/10.2217/nnm.13.50
111. Hudecki A, Kiryczyński G, Łos MJ (2019) Biomaterials, definition, overview. In: Stem cells and biomaterials for regenerative medicine. Elsevier, Amsterdam, pp 85–98. https://doi.org/10.1016/B978-0-12-812258-7.00007-1
112. Bhardwaj N, Chouhan D, Mandal BB (2018) 3D functional scaffolds for skin tissue engineering. In: Functional 3D tissue engineering scaffolds. Elsevier, Amsterdam, pp 345–365. https://doi.org/10.1016/B978-0-08-100979-6.00014-8
113. O'Brien FJ (2011) Biomaterials & scaffolds for tissue engineering. Mater Today 14(3):88–95. https://doi.org/10.1016/S1369-7021(11)70058-X

114. Balint R, Cassidy NJ, Cartmell SH (2014) Conductive polymers: towards a smart biomaterial for tissue engineering. Acta Biomater 10(6):2341–2353. https://doi.org/10.1016/j.actbio.2014.02.015
115. Gupta P et al (2016) Mimicking form and function of native small diameter vascular conduits using mulberry and non-mulberry patterned silk films. ACS Appl Mater Interfaces 8(25):15874–15888. https://doi.org/10.1021/acsami.6b00783
116. Chan BP, Leong KW (2008) Scaffolding in tissue engineering: general approaches and tissue-specific considerations. Eur Spine J 17(S4):467–479. https://doi.org/10.1007/s00586-008-0745-3
117. Rahmati M, Blaker JJ, Lyngstadaas SP, Mano JF, Haugen HJ (2020) Designing multigradient biomaterials for skin regeneration. Mater Today Adv 5:100051. https://doi.org/10.1016/j.mtadv.2019.100051
118. Jeong K-H, Park D, Lee Y-C (2017) Polymer-based hydrogel scaffolds for skin tissue engineering applications: a mini-review. J Polym Res 24(7):112. https://doi.org/10.1007/s10965-017-1278-4
119. Bernard A, Renault JP, Michel B, Bosshard HR, Delamarche E (2000) Microcontact printing of proteins. Adv Mater. https://doi.org/10.1002/1521-4095(200007)12:14<1067::AID-ADMA1067>3.0.CO;2-M
120. Therriault D, White SR, Lewis JA (2003) Chaotic mixing in three-dimensional microvascular networks fabricated by direct-write assembly. Nat Mater. https://doi.org/10.1038/nmat863
121. Wang Z, Samanipour R, Koo KI, Kim K (2015) Organ-on-a-chip platforms for drug delivery and cell characterization: a review. Sens Mater. https://doi.org/10.18494/SAM.2015.1086
122. Sticker D, Rothbauer M, Lechner S, Hehenberger MT, Ertl P (2015) Multi-layered, membrane-integrated microfluidics based on replica molding of a thiol-ene epoxy thermoset for organ-on-a-chip applications. Lab Chip. https://doi.org/10.1039/c5lc01028d
123. Lee J, Cuddihy MJ, Kotov NA (2008) Three-dimensional cell culture matrices: state of the art. Tissue Eng B Rev. https://doi.org/10.1089/teb.2007.0150
124. Okugawa Y, Hirai Y (2013) Extracellular epimorphin modulates epidermal differentiation signals mediated by epidermal growth factor receptor. J Dermatol Sci. https://doi.org/10.1016/j.jdermsci.2012.11.006
125. Hern DL, Hubbell JA (1998) Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. J Biomed Mater Res. https://doi.org/10.1002/(SICI)1097-4636(199802)39:2<266::AID-JBM14>3.0.CO;2-B
126. Hosseinkhani H et al (2013) Engineering three-dimensional collagen-IKVAV matrix to mimic neural microenvironment. ACS Chem Neurosci. https://doi.org/10.1021/cn400075h
127. Zhao X et al (2016) Photocrosslinkable gelatin hydrogel for epidermal tissue engineering. Adv Healthc Mater. https://doi.org/10.1002/adhm.201500005
128. Fu L, Xie J, Carlson MA, Reilly DA (2017) Three-dimensional nanofiber scaffolds with arrayed holes for engineering skin tissue constructs. MRS Commun. https://doi.org/10.1557/mrc.2017.49
129. Pan H, Jiang H, Chen W (2006) Interaction of dermal fibroblasts with electrospun composite polymer scaffolds prepared from dextran and poly lactide-co-glycolide. Biomaterials. https://doi.org/10.1016/j.biomaterials.2006.01.032
130. Plaza C, Meyrignac C, Botto J-M, Capallere C (2021) Characterization of a new full-thickness *in vitro* skin model. Tissue Eng Part C Methods 27(7):411–420. https://doi.org/10.1089/ten.tec.2021.0035
131. Xia Y, Whitesides GM (1998) Soft lithography. Annu Rev Mater Sci. https://doi.org/10.1146/annurev.matsci.28.1.153
132. Qin D, Xia Y, Whitesides GM (2010) Soft lithography for micro- and nanoscale patterning. Nat Protoc 5(3):491–502. https://doi.org/10.1038/nprot.2009.234
133. Kim JJ et al (2019) A microscale, full-thickness, human skin on a chip assay simulating neutrophil responses to skin infection and antibiotic treatments. Lab Chip 19(18):3094–3103. https://doi.org/10.1039/C9LC00399A

134. Kwak BS, Jin SP, Kim SJ, Kim EJ, Chung JH, Sung JH (2020) Microfluidic skin chip with vasculature for recapitulating the immune response of the skin tissue. Biotechnol Bioeng 117(6):1853–1863. https://doi.org/10.1002/bit.27320
135. Warrick J, Meyvantsson I, Ju J, Beebe DJ (2007) High-throughput microfluidics: improved sample treatment and washing over standard wells. Lab Chip. https://doi.org/10.1039/b613350a
136. Dittrich PS, Manz A (2006) Lab-on-a-chip: microfluidics in drug discovery. Nat Rev Drug Discov. https://doi.org/10.1038/nrd1985
137. Valencia L et al (2021) A new microfluidic method enabling the generation of multi-layered tissues-on-chips using skin cells as a proof of concept. Sci Rep 11(1):13160. https://doi.org/10.1038/s41598-021-91875-z
138. Jeon HM, Kim K, Choi KC, Sung GY (2020) Side-effect test of sorafenib using 3-D skin equivalent based on microfluidic skin-on-a-chip. J Ind Eng Chem 82:71–80. https://doi.org/10.1016/j.jiec.2019.09.044
139. Kim K, Jeon HM, Choi KC, Sung GY (2020) Testing the effectiveness of curcuma longa leaf extract on a skin equivalent using a pumpless skin-on-a-chip model. IJMS 21(11):3898. https://doi.org/10.3390/ijms21113898
140. Sasaki N, Tsuchiya K, Kobayashi H (2019) Photolithography-free skin-on-a-chip for parallel permeation assays. Sens Mater 31(1):9
141. Lim HY et al (2018) Development of wrinkled skin-on-a-chip (WSOC) by cyclic uniaxial stretching. J Ind Eng Chem 68:238–245. https://doi.org/10.1016/j.jiec.2018.07.050
142. Song HJ et al (2018) Development of 3D skin-equivalent in a pump-less microfluidic chip. J Ind Eng Chem 60:355–359. https://doi.org/10.1016/j.jiec.2017.11.022
143. Song HJ, Lim HY, Chun W, Choi KC, Sung JH, Sung GY (2017) Fabrication of a pumpless, microfluidic skin chip from different collagen sources. J Ind Eng Chem 56:375–381. https://doi.org/10.1016/j.jiec.2017.07.034
144. Alexander F, Eggert S, Wiest J (2018) Skin-on-a-chip: transepithelial electrical resistance and extracellular acidification measurements through an automated air-liquid interface. Genes 9(2):114. https://doi.org/10.3390/genes9020114
145. Maschmeyer I et al (2015) Chip-based human liver–intestine and liver–skin co-cultures – a first step toward systemic repeated dose substance testing in vitro. Eur J Pharm Biopharm 95:77–87. https://doi.org/10.1016/j.ejpb.2015.03.002
146. Wagner I et al (2013) A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture. Lab Chip 13(18):3538. https://doi.org/10.1039/c3lc50234a
147. Calcein Am - an overview. ScienceDirect Topics. https://www.sciencedirect.com/topics/chemistry/calcein-am. Accessed 07 Nov 2021
148. Ethidium Homodimer - an overview. ScienceDirect Topics. https://www.sciencedirect.com/topics/chemistry/ethidium-homodimer. Accessed 07 Nov 2021
149. Crowley LC, Scott AP, Marfell BJ, Boughaba JA, Chojnowski G, Waterhouse NJ (2016) Measuring cell death by propidium iodide uptake and flow cytometry. Cold Spring Harb Protoc 2016(7). https://doi.org/10.1101/pdb.prot087163
150. Viability Assay - an overview. ScienceDirect Topics. https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/viability-assay. Accessed 07 Nov 2021
151. Moniz T, Lima SAC, Reis S (2020) Human skin models: from healthy to disease-mimetic systems; characteristics and applications. Br J Pharmacol 177(19):4314–4329. https://doi.org/10.1111/bph.15184
152. Souto LRM, Vassallo J, Rehder J, Pinto GA, Puzzi MB (2009) Immunoarchitectural characterization of a human skin model reconstructed in vitro. Sao Paulo Med J 127(1):28–33. https://doi.org/10.1590/S1516-31802009000100007
153. Barker CL et al (2004) The development and characterization of an in vitro model of psoriasis. J Investig Dermatol 123(5):892–901. https://doi.org/10.1111/j.0022-202X.2004.23435.x

154. Grimm J, Kircher MF, Weissleder R (2007) Cell tracking. Principles and applications. Radiologe 47(1):25–33. https://doi.org/10.1007/s00117-006-1449-5
155. Halabi EA, Arasa J, Püntener S, Collado-Diaz V, Halin C, Rivera-Fuentes P (2020) Dual-activatable cell tracker for controlled and prolonged single-cell labeling. ACS Chem Biol 15(6):1613–1620. https://doi.org/10.1021/acschembio.0c00208
156. Liang X, Graf BW, Boppart SA (2011) In vivo multiphoton microscopy for investigating biomechanical properties of human skin. Cell Mol Bioeng 4(2):231–238. https://doi.org/10.1007/s12195-010-0147-6
157. Pawlaczyk M, Lelonkiewicz M, Wieczorowski M (2013) Age-dependent biomechanical properties of the skin. Postepy Dermatol Allergol 30(5):302–306. https://doi.org/10.5114/pdia.2013.38359
158. Joodaki H, Panzer MB (2018) Skin mechanical properties and modeling: a review. https://journals.sagepub.com/doi/10.1177/0954411918759801. Accessed 07 Nov 2021
159. Jachowicz J, McMullen R, Prettypaul D (2007) Indentometric analysis of in vivo skin and comparison with artificial skin models. Skin Res Technol 13(3):299–309. https://doi.org/10.1111/j.1600-0846.2007.00229.x
160. Sutterby E, Thurgood P, Baratchi S, Khoshmanesh K, Pirogova E (2020) Microfluidic skin-on-a-chip models: toward biomimetic artificial skin. Small 16(39):2002515. https://doi.org/10.1002/smll.202002515
161. Mechanical properties and Young's modulus of human skin in vivo. SpringerLink. https://link.springer.com/article/10.1007/BF00406415. Accessed 07 Nov 2021
162. Kalra A, Lowe A (2016) Mechanical behaviour of skin: a review. J Mater Sci Eng 5. https://doi.org/10.4172/2169-0022.1000254
163. Dimitrow E et al (2009) Sensitivity and specificity of multiphoton laser tomography for in vivo and ex vivo diagnosis of malignant melanoma. J Invest Dermatol 129(7):1752–1758. https://doi.org/10.1038/jid.2008.439
164. Koehler MJ, König K, Elsner P, Bückle R, Kaatz M (2006) In vivo assessment of human skin aging by multiphoton laser scanning tomography. Opt Lett 31(19):2879–2881. https://doi.org/10.1364/ol.31.002879
165. Konig K, Ehlers A, Stracke F, Riemann I (2006) In vivo drug screening in human skin using femtosecond laser multiphoton tomography. Skin Pharmacol Physiol 19(2):78–88. https://doi.org/10.1159/000091974
166. Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, Hickman JJ (2015) TEER measurement techniques for in vitro barrier model systems. J Lab Autom 20(2):107–126. https://doi.org/10.1177/2211068214561025
167. Alberti M et al (2017) Multi-chamber microfluidic platform for high-precision skin permeation testing. Lab Chip 17(9):1625–1634. https://doi.org/10.1039/c6lc01574c
168. Netzlaff F, Lehr C-M, Wertz PW, Schaefer UF (2005) The human epidermis models EpiSkin, SkinEthic and EpiDerm: an evaluation of morphology and their suitability for testing phototoxicity, irritancy, corrosivity, and substance transport. Eur J Pharm Biopharm 60(2):167–178. https://doi.org/10.1016/j.ejpb.2005.03.004
169. Alexander FA, Eggert S, Wiest J (2018) Skin-on-a-chip: transepithelial electrical resistance and extracellular acidification measurements through an automated air-liquid interface. Genes (Basel) 9(2):E114. https://doi.org/10.3390/genes9020114
170. Niehues H, Bouwstra JA, Ghalbzouri AE, Brandner JM, Zeeuwen PLJM, van den Bogaard EH (2018) 3D skin models for 3R research: the potential of 3D reconstructed skin models to study skin barrier function. Exp Dermatol 27(5):501–511. https://doi.org/10.1111/exd.13531
171. Pauty J et al (2017) A vascular permeability assay using an in vitro human microvessel model mimicking the inflammatory condition. Nano 1(1):103–113. https://doi.org/10.7150/ntno.18303
172. Lukács B et al (2019) Skin-on-a-chip device for ex vivo monitoring of transdermal delivery of drugs—design, fabrication, and testing. Pharmaceutics 11(9):445. https://doi.org/10.3390/pharmaceutics11090445

173. Bajza Á et al (2020) Verification of p-glycoprotein function at the dermal barrier in diffusion cells and dynamic 'skin-on-a-chip' microfluidic device. Pharmaceutics 12(9):1–16. https://doi.org/10.3390/pharmaceutics12090804
174. Rousselle P, Gentilhomme E, Neveux Y (2017) Markers of Epidermal Proliferation and Differentiation. In: Humbert P, Fanian F, Maibach HI, Agache P (eds) Agache's measuring the skin: non-invasive investigations, physiology, normal constants. Springer, Cham, pp 407–415. https://doi.org/10.1007/978-3-319-32383-1_37
175. Ponec M, Boelsma E, Weerheim A, Mulder A, Bouwstra J, Mommaas M (2000) Lipid and ultrastructural characterization of reconstructed skin models. Int J Pharm 203(1):211–225. https://doi.org/10.1016/S0378-5173(00)00459-2
176. Poumay Y, Coquette A (2006) Modelling the human epidermis in vitro: tools for basic and applied research. Arch Dermatol Res 298(8):361–369. https://doi.org/10.1007/s00403-006-0709-6
177. Kuempel D, Swartzendruber DC, Squier CA, Wertz PW (1998) In vitro reconstitution of stratum corneum lipid lamellae. Biochim Biophys Acta Biomembr 1372(1):135–140. https://doi.org/10.1016/S0005-2736(98)00053-4
178. Bataillon M et al (2019) Characterization of a new reconstructed full thickness skin model, T-Skin™, and its application for investigations of anti-aging compounds. Int J Mol Sci 20(9):9. https://doi.org/10.3390/ijms20092240
179. Marionnet C, Pierrard C, Vioux-Chagnoleau C, Sok J, Asselineau D, Bernerd F (2006) Interactions between fibroblasts and keratinocytes in morphogenesis of dermal epidermal junction in a model of reconstructed skin. J Investig Dermatol 126(5):971–979. https://doi.org/10.1038/sj.jid.5700230
180. Krieg T, Aumailley M (2011) The extracellular matrix of the dermis: flexible structures with dynamic functions. Exp Dermatol 20(8):689–695. https://doi.org/10.1111/j.1600-0625.2011.01313.x
181. Supp DM et al (2019) Collagen VII expression is required in both keratinocytes and fibroblasts for anchoring fibril formation in bilayer engineered skin substitutes. Cell Transplant 28(9–10):1242–1256. https://doi.org/10.1177/0963689719857657
182. Lippens S et al (2004) Vitamin D3 induces caspase-14 expression in psoriatic lesions and enhances caspase-14 processing in organotypic skin cultures. Am J Pathol 165(3):833–841. https://doi.org/10.1016/S0002-9440(10)63346-9
183. Chiricozzi A et al (2014) IL-17 induces an expanded range of downstream genes in reconstituted human epidermis model. PLoS One 9(2):e90284. https://doi.org/10.1371/journal.pone.0090284
184. Yin X, Zhang S, Li B, Zhang Y, Zhang X (2019) IL28RA inhibits human epidermal keratinocyte proliferation by inhibiting cell cycle progression. Mol Biol Rep 46(1):1189–1197. https://doi.org/10.1007/s11033-019-04586-0
185. Gibbs S et al (2013) An epidermal equivalent assay for identification and ranking potency of contact sensitizers. Toxicol Appl Pharmacol 272(2):529–541. https://doi.org/10.1016/j.taap.2013.07.003
186. Saito K, Takenouchi O, Nukada Y, Miyazawa M, Sakaguchi H (2017) An in vitro skin sensitization assay termed EpiSensA for broad sets of chemicals including lipophilic chemicals and pre/pro-haptens. Toxicol In Vitro 40:11–25. https://doi.org/10.1016/j.tiv.2016.12.005
187. Facy V, Flouret V, Régnier M, Schmidt R (2005) Reactivity of Langerhans cells in human reconstructed epidermis to known allergens and UV radiation. Toxicol In Vitro 19(6):787–795. https://doi.org/10.1016/j.tiv.2005.03.018
188. De Vuyst É et al (2018) Methyl-β-cyclodextrin concurs with interleukin (IL)-4, IL-13 and IL-25 to induce alterations reminiscent of atopic dermatitis in reconstructed human epidermis. Exp Dermatol 27(4):435–437. https://doi.org/10.1111/exd.13113

189. TNF-α and Th2 cytokines induce atopic dermatitis–like features on epidermal differentiation proteins and stratum corneum lipids in human skin equivalents. J Invest Dermatol. https://www.jidonline.org/article/S0022-202X(15)36873-1/fulltext. Accessed 20 Feb 2021
190. Boukhedouni N et al (2020) Type-1 cytokines regulate MMP-9 production and E-cadherin disruption to promote melanocyte loss in vitiligo. JCI Insight 5(11). https://doi.org/10.1172/jci.insight.133772
191. Matei A-E et al (2019) Vascularised human skin equivalents as a novel in vitro model of skin fibrosis and platform for testing of antifibrotic drugs. Ann Rheum Dis 78(12):1686–1692. https://doi.org/10.1136/annrheumdis-2019-216108
192. Hill DS et al (2015) A novel fully humanized 3D skin equivalent to model early melanoma invasion. Mol Cancer Ther 14(11):2665–2673. https://doi.org/10.1158/1535-7163.MCT-15-0394
193. Vörsmann H et al (2013) Development of a human three-dimensional organotypic skin-melanoma spheroid model for in vitro drug testing. Cell Death Dis 4(7):7. https://doi.org/10.1038/cddis.2013.249
194. Browning JR et al (2020) A 3D biofabricated cutaneous squamous cell carcinoma tissue model with multi-channel confocal microscopy imaging biomarkers to quantify antitumor effects of chemotherapeutics in tissue. Oncotarget 11(27):2587–2596. https://doi.org/10.18632/oncotarget.27570
195. Linde N, Gutschalk CM, Hoffmann C, Yilmaz D, Mueller MM (2012) Integrating macrophages into organotypic co-cultures: a 3D in vitro model to study tumor-associated macrophages. PLoS One 7(7):e40058. https://doi.org/10.1371/journal.pone.0040058
196. Obrigkeit DH et al (2009) Effects of photodynamic therapy evaluated in a novel three-dimensional squamous cell carcinoma organ construct of the skin. Photochem Photobiol 85(1):272–278. https://doi.org/10.1111/j.1751-1097.2008.00432.x
197. Tan JJY, Common JE, Wu C, Ho PCL, Kang L (2019) Keratinocytes maintain compartmentalization between dermal papilla and fibroblasts in 3D heterotypic tri-cultures. Cell Prolif 52(5):e12668. https://doi.org/10.1111/cpr.12668
198. Lin B et al (2016) Surface tension guided hanging-drop: producing controllable 3D spheroid of high-passaged human dermal papilla cells and forming inductive microtissues for hair-follicle regeneration. ACS Appl Mater Interfaces 8(9):5906–5916. https://doi.org/10.1021/acsami.6b00202
199. Gupta AC et al (2018) Establishment of an in vitro organoid model of dermal papilla of human hair follicle. J Cell Physiol 233(11):9015–9030. https://doi.org/10.1002/jcp.26853
200. de Breij A et al (2012) Three-dimensional human skin equivalent as a tool to study Acinetobacter baumannii colonization. Antimicrob Agents Chemother 56(5):2459–2464. https://doi.org/10.1128/AAC.05975-11
201. Havlikova J, May RC, Styles IB, Cooper HJ (2020) Direct identification of bacterial and human proteins from infected wounds in living 3D skin models. Sci Rep 10(1):1. https://doi.org/10.1038/s41598-020-68233-6
202. Liang P-P et al (2016) A trichophyton rubrum infection model based on the reconstructed human epidermis - Episkin®. Chin Med J 129(1):54–58. https://doi.org/10.4103/0366-6999.172573
203. Faway É, Cambier L, Mignon B, Poumay Y, Lambert de Rouvroit C (2017) Modeling dermatophytosis in reconstructed human epidermis: a new tool to study infection mechanisms and to test antifungal agents. Med Mycol 55(5):485–494. https://doi.org/10.1093/mmy/myw111
204. Coolen NA, Vlig M, Bogaerdt AJVD, Middelkoop E, Ulrich MMW (2008) Development of an in vitro burn wound model. Wound Repair Regen 16(4):559–567. https://doi.org/10.1111/j.1524-475X.2008.00403.x
205. Weinmüllner R et al (2020) Organotypic human skin culture models constructed with senescent fibroblasts show hallmarks of skin aging. NPJ Aging Mech Dis 6(1):1–7. https://doi.org/10.1038/s41514-020-0042-x

206. Dos Santos M, Metral E, Boher A, Rousselle P, Thepot A, Damour O (2015) In vitro 3-D model based on extending time of culture for studying chronological epidermis aging. Matrix Biol 47:85–97. https://doi.org/10.1016/j.matbio.2015.03.009
207. Bernerd F, Asselineau D (1998) UVA exposure of human skin reconstructed in vitro induces apoptosis of dermal fibroblasts: subsequent connective tissue repair and implications in photoaging. Cell Death Differ 5(9):9. https://doi.org/10.1038/sj.cdd.4400413
208. Meloni M, Farina A, de Servi B (2010) Molecular modifications of dermal and epidermal biomarkers following UVA exposures on reconstructed full-thickness human skin. Photochem Photobiol Sci 9(4):439–447. https://doi.org/10.1039/B9PP00164F
209. Karapetsas A et al (2020) Honey extracts exhibit cytoprotective properties against UVB-induced photodamage in human experimental skin models. Antioxidants 9(7):7. https://doi.org/10.3390/antiox9070566
210. Verdin A et al (2019) An in vitro model to evaluate the impact of environmental fine particles (PM0.3-2.5) on skin damage. Toxicol Lett 305:94–102. https://doi.org/10.1016/j.toxlet.2019.01.016
211. McKenzie NE (2005) The drug development process. Fundament Cancer Prev:131–138. https://doi.org/10.1007/3-540-26337-3_7
212. Drews J (2000) Drug discovery: a historical perspective. Science. https://doi.org/10.1126/science.287.5460.1960
213. Sung JH et al (2013) Microfabricated mammalian organ systems and their integration into models of whole animals and humans. Lab Chip. https://doi.org/10.1039/c3lc41017j
214. Luni C, Serena E, Elvassore N (2014) Human-on-chip for therapy development and fundamental science. Curr Opin Biotechnol. https://doi.org/10.1016/j.copbio.2013.08.015
215. Jin H, Yu Y (2016) A review of the application of body-on-a-chip for drug test and its latest trend of incorporating barrier tissue. J Lab Autom. https://doi.org/10.1177/2211068215619126
216. Biglari S et al (2019) Simulating inflammation in a wound microenvironment using a dermal wound-on-a-chip model. Adv Healthc Mater. https://doi.org/10.1002/adhm.201801307
217. Ray TR et al (2021) Soft, skin-interfaced sweat stickers for cystic fibrosis diagnosis and management. Sci Transl Med. https://doi.org/10.1126/scitranslmed.abd8109
218. Can sweat shed a light on disease severity in atopic dermatitis? [Online]. Available: https://www.dermatologytimes.com/view/can-sweat-shed-light-disease-severity-atopic-dermatitis. Accessed 30 July 2021
219. Sheibani S et al (2021) Extended gate field-effect-transistor for sensing cortisol stress hormone. Commun Mater. https://doi.org/10.1038/s43246-020-00114-x
220. Kim S et al (2020) Soft, skin-interfaced microfluidic systems with integrated immunoassays, fluorometric sensors, and impedance measurement capabilities. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.2012700117
221. Gentag Announces Lab-on-a-Chip (LOC) Patent For Diagnostics With Cell Phones (NFC) | Gentag. [Online]. Available: https://gentag.com/gentags-lab-chip-loc-patent-diagnostics-cell-phones-issues/. Accessed 30 July 2021
222. Gorjikhah F et al (2016) Improving 'lab-on-a-chip' techniques using biomedical nanotechnology: a review. Artif Cells Nanomed Biotechnol 44(7):1609–1614. https://doi.org/10.3109/21691401.2015.1129619
223. Researchers are developing a 'Lab-on-skin' to monitor biomarkers • healthcare-in-europe.com. [Online]. Available: https://healthcare-in-europe.com/en/news/researchers-are-developing-a-lab-on-skin-to-monitor-biomarkers.html. Accessed 30 July 2021
224. Tavares RSN et al (2020) Skin irritation testing beyond tissue viability: fucoxanthin effects on inflammation, homeostasis, and metabolism. Pharmaceutics. https://doi.org/10.3390/pharmaceutics12020136
225. Wang XP et al (2004) The interleukin-6 cytokine system regulates epidermal permeability barrier homeostasis. J Investig Dermatol. https://doi.org/10.1111/j.0022-202X.2004.22736.x

226. Jensen JM, Schütze S, Förl M, Krönke M, Proksch E (1999) Roles for tumor necrosis factor receptor p55 and sphingomyelinase in repairing the cutaneous permeability barrier. J Clin Investig. https://doi.org/10.1172/JCI5307
227. Kim J, Kim K, Sung GY (2020) Coenzyme q10 efficacy test for human skin equivalents using a pumpless skin-on-a-chip system. Int J Mol Sci. https://doi.org/10.3390/ijms21228475
228. Kim K, Jeon HM, Choi KC, Sung GY (2020) Testing the effectiveness of curcuma longa leaf extract on a skin equivalent using a pumpless skin-on-a-chip model. Int J Mol Sci 21(11):1–15. https://doi.org/10.3390/ijms21113898
229. Pauty J et al (2021) A 3D tissue model-on-a-chip for studying the effects of human senescent fibroblasts on blood vessels. Biomater Sci. https://doi.org/10.1039/d0bm01297a
230. Ruze A et al (2018) Low shear stress upregulates the expression of fractalkine through the activation of mitogen-activated protein kinases in endothelial cells. Blood Coagul Fibrinolysis. https://doi.org/10.1097/MBC.0000000000000701
231. Lin JY, Lo KY, Sun YS (2019) A microfluidics-based wound-healing assay for studying the effects of shear stresses, wound widths, and chemicals on the wound-healing process. Sci Rep. https://doi.org/10.1038/s41598-019-56753-9
232. Lawrence BJ, Madihally SV (2008) Cell colonization in degradable 3D porous matrices. Cell Adhes Migr. https://doi.org/10.4161/cam.2.1.5884
233. Suwannaphan T et al (2016) Investigation of shear stress and cell survival in a microfluidic chip for a single cell study. https://doi.org/10.1109/BMEiCON.2015.7399541
234. Agarwal T, Narayana GH, Banerjee I (2019) Keratinocytes are mechanoresponsive to the microflow-induced shear stress. Cytoskeleton. https://doi.org/10.1002/cm.21521
235. Allwardt V et al (2020) Translational roadmap for the organs-on-a-chip industry toward broad adoption. Bioengineering 7(3):3. https://doi.org/10.3390/bioengineering7030112
236. How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov. https://www.nature.com/articles/nrd3078. Accessed 09 July 2021
237. Harrison RK (2016) Phase II and phase III failures: 2013–2015. Nat Rev Drug Discov 15(12): 817–818. https://doi.org/10.1038/nrd.2016.184
238. Arrowsmith J, Miller P (2013) Phase II and Phase III attrition rates 2011–2012. Nat Rev Drug Discov 12(8):569–569. https://doi.org/10.1038/nrd4090
239. Probst C, Schneider S, Loskill P (2018) High-throughput organ-on-a-chip systems: current status and remaining challenges. Curr Opin Biomed Eng 6:33–41. https://doi.org/10.1016/j.cobme.2018.02.004
240. Reardon S (2015) 'Organs-on-chips' go mainstream. Nature 523(7560):266–266. https://doi.org/10.1038/523266a
241. DiMasi JA, Grabowski HG, Hansen RW (2016) Innovation in the pharmaceutical industry: new estimates of R&D costs. J Health Econ 47:20–33. https://doi.org/10.1016/j.jhealeco.2016.01.012
242. Prasad V, Mailankody S (2017) Research and development spending to bring a single cancer drug to market and revenues after approval. JAMA Intern Med 177(11):1569–1575. https://doi.org/10.1001/jamainternmed.2017.3601
243. Impact of organ-on-a-chip technology on pharmaceutical R&D costs. Elsevier Enhanced Reader. https://reader.elsevier.com/reader/sd/pii/S135964461930042X?token=9AC2BDA79D1EAD5E80268995FCBD5A3FE36048296F31BCBD44426678C8AFBDDC501CAF4B652426D61A2B3300A7670A7F&originRegion=eu-west-1&originCreation=20210708174208. Accessed 09 July 2021
244. C. for D. E. and Research. Office of Clinical Pharmacology, FDA, 31 Mar 2021. https://www.fda.gov/about-fda/center-drug-evaluation-and-research-cder/office-clinical-pharmacology. Accessed 09 July 2021
245. C. for D. E. and Research. Product Development Under the Animal Rule. U.S. Food and Drug Administration, 14 Apr 2020. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/product-development-under-animal-rule. Accessed 09 July 2021

246. OECD (2019) Test No. 431: In vitro skin corrosion: reconstructed human epidermis (RHE) test method. Organisation for Economic Co-operation and Development, Paris. Available: https://www.oecd-ilibrary.org/environment/test-no-431-in-vitro-skin-corrosion-reconstructed-human-epidermis-rhe-test-method_9789264264618-en. Accessed 07 Nov 2021
247. OECD (2010) Test No. 439: In vitro skin irritation: reconstructed human epidermis test method. Organisation for Economic Co-operation and Development, Paris. Available: https://www.oecd-ilibrary.org/environment/test-no-439-in-vitro-skin-irritation_9789264090958-en. Accessed 07 Nov 2021
248. OECD (2021) Test No. 498: In vitro phototoxicity - Reconstructed Human Epidermis Phototoxicity test method. Organisation for Economic Co-operation and Development, Paris. Available: https://www.oecd-ilibrary.org/environment/test-no-498-in-vitro-phototoxicity-reconstructed-human-epidermis-phototoxicity-test-method_7b2f9ea0-en. Accessed 07 Nov 2021
249. Pasman T, Grijpma D, Stamatialis D, Poot A (2018) Flat and microstructured polymeric membranes in organs-on-chips. J R Soc Interface. https://doi.org/10.1098/rsif.2018.0351
250. Huh D (2015) A human breathing lung-on-a-chip. Ann Am Thorac Soc. https://doi.org/10.1513/AnnalsATS.201410-442MG
251. Gumuscu B, Albers HJ, Van Den Berg A, Eijkel JCT, Van Der Meer AD (2017) Compartmentalized 3D tissue culture arrays under controlled microfluidic delivery. Sci Rep. https://doi.org/10.1038/s41598-017-01944-5
252. Richardson L, Jeong S, Kim S, Hart A, Menon R (2019) Amnion membrane organ-on-chip: an innovative approach to study cellular interactions. FASEB J. https://doi.org/10.1096/fj.201900020RR
253. van Meer BJ et al (2017) Small molecule absorption by PDMS in the context of drug response bioassays. Biochem Biophys Res Commun. https://doi.org/10.1016/j.bbrc.2016.11.062
254. Toepke MW, Beebe DJ (2006) PDMS absorption of small molecules and consequences in microfluidic applications. Lab Chip 6(12):12. https://doi.org/10.1039/B612140C
255. Berthier E, Young EWK, Beebe D (2012) Engineers are from PDMS-land, biologists are from polystyrenia. Lab Chip. https://doi.org/10.1039/c2lc20982a
256. Eddington DT, Puccinelli JP, Beebe DJ (2006) Thermal aging and reduced hydrophobic recovery of polydimethylsiloxane. Sensors Actuators B Chem. https://doi.org/10.1016/j.snb.2005.04.037
257. Agarwal A, Farouz Y, Nesmith AP, Deravi LF, McCain ML, Parker KK (2013) Micropatterning alginate substrates for in vitro cardiovascular muscle on a chip. Adv Funct Mater. https://doi.org/10.1002/adfm.201203319
258. Ren K, Zhou J, Wu H (2013) Materials for microfluidic chip fabrication. Acc Chem Res. https://doi.org/10.1021/ar300314s
259. Borysiak MD, Bielawski KS, Sniadecki NJ, Jenkel CF, Vogt BD, Posner JD (2013) Simple replica micromolding of biocompatible styrenic elastomers. Lab Chip. https://doi.org/10.1039/c3lc50426c
260. Wikswo JP (2017) Looking to the future of organs-on-chips: interview with professor John Wikswo. Future Sci OA. https://doi.org/10.4155/fsoa-2016-0085
261. Henry OYF, Villenave R, Cronce MJ, Leineweber WD, Benz MA, Ingber DE (2017) Organs-on-chips with integrated electrodes for trans-epithelial electrical resistance (TEER) measurements of human epithelial barrier function. Lab Chip. https://doi.org/10.1039/c7lc00155j
262. Rennert K et al (2015) A microfluidically perfused three dimensional human liver model. Biomaterials. https://doi.org/10.1016/j.biomaterials.2015.08.043
263. Gruber P, Marques MPC, Szita N, Mayr T (2017) Integration and application of optical chemical sensors in microbioreactors. Lab Chip. https://doi.org/10.1039/c7lc00538e
264. Wikswo JP et al (2013) Engineering challenges for instrumenting and controlling integrated organ-on-chip systems. IEEE Trans Biomed Eng. https://doi.org/10.1109/TBME.2013.2244891

265. Gordon S et al (2015) Non-animal models of epithelial barriers (skin, intestine and lung) in research, industrial applications and regulatory toxicology. ALTEX 32(4):327–378. https://doi.org/10.14573/altex.1510051
266. Wainwright DJ (1995) Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. Burns 21(4):243–248. https://doi.org/10.1016/0305-4179(95)93866-i
267. Boyce ST, Kagan RJ, Meyer NA, Yakuboff KP, Warden GD (1999) The 1999 clinical research award. Cultured skin substitutes combined with Integra Artificial Skin to replace native skin autograft and allograft for the closure of excised full-thickness burns. J Burn Care Rehabil 20(6):453–461. https://doi.org/10.1097/00004630-199920060-00006
268. Eaglstein WH, Falanga V (1997) Tissue engineering and the development of Apligraf, a human skin equivalent. Clin Ther 19(5):894–905. https://doi.org/10.1016/s0149-2918(97)80043-4
269. Hansbrough JF, Mozingo DW, Kealey GP, Davis M, Gidner A, Gentzkow GD (1997) Clinical trials of a biosynthetic temporary skin replacement, dermagraft-transitional covering, compared with cryopreserved human cadaver skin for temporary coverage of excised burn wounds. J Burn Care Rehabil 18(1 Pt 1):43–51. https://doi.org/10.1097/00004630-199701000-00008
270. Vig K et al (2017) Advances in skin regeneration using tissue engineering. Int J Mol Sci 18(4):E789. https://doi.org/10.3390/ijms18040789
271. Centanni JM et al (2011) StrataGraft skin substitute is well-tolerated and is not acutely immunogenic in patients with traumatic wounds: results from a prospective, randomized, controlled dose escalation trial. Ann Surg 253(4):672–683. https://doi.org/10.1097/SLA.0b013e318210f3bd
272. Allen-Hoffmann B, Rooney P (2016) Current innovations for the treatment of chronic wounds, pp 265–287. https://doi.org/10.1016/B978-0-12-801654-1.00013-9

Organs-on-a-Chip in Preclinical Studies

23

Surat Parvatam and Pavithra L. Chavali

Abstract

The scientific community is becoming increasingly aware that animal models frequently fail to capture the complexities of human biology. Organ-on-chip (OoC) technology has advanced rapidly over the last decade as a result of advances in several fields, including microfluidics, cell biology, bioengineering, physiology, and computational biology. These microfluidic devices are intended to mimic and recapitulate the essential physiology and functions of a single human organ or a network of organs. The history and design principles of organ-on-chip devices are discussed in this chapter, followed by advances in understanding human development and disease using these microfluidic devices. We also discuss how pharmacokinetic-pharmacodynamic analysis on chips could be the next frontier in drug discovery and testing. Towards the end, various challenges that still need to be addressed in order for these systems to be widely adopted and used, such as validation, cell sourcing, platform fabrication, and defining context-of-use across different sectors and globally, are discussed.

Keywords

Organ-on-chip · Microfluidic device · Microsystem · Disease modelling · Inflammatory

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23.1 Introduction

Currently, preclinical drug testing is based on a paradigm that involves testing high-doses of molecules in animal models. However, due to the vast biological differences between animals and humans, this paradigm is fraught with limitations. While mice and humans share genetic similarities in 70% of protein coding regions, there are significant differences that contribute to variations in the stress response, drug metabolism, immune pathways, and so on. According to research, while similar groups of genes are involved in burn, trauma, and infection in humans, distinct sets of genes are involved in mice to fight different types of stress [1]. Mice are also more resistant to infection; a lethal dose of endotoxin in mice is 5–25 mg/kg, while $<$30 ng/kg in humans can cause shock [2]. Species-specific differences in various drug metabolising enzymes, such as Cytochrome P, also contribute to differences in how drugs are absorbed, distributed, metabolised, and transported in animals and humans [3]. Furthermore, there is a lack of organism-specific information on how biological pathways are differently regulated between animals and humans, raising questions about the suitability of using different animals to model human pathways or diseases [4]. All of this has resulted in the high failure rate of the current drug discovery paradigm, with studies revealing that 8 out of 9 drugs that pass through the drug discovery pipeline fail during the clinical trials. This failure has been attributed to low precision rates in identification of relevant molecules during the drug discovery process [5]. These drawbacks have shifted the focus of the global scientific community away from the animal models towards model systems that can better represent the complexities of human biology. Two-dimensional in vitro cultures, which are also used in preclinical stages of drug development, do not mimic the cell behaviours and functions that are dictated by micro-environmental cues, such as neighbouring cells, extracellular matrix, soluble factors, and physical forces, among others. This limits their physiological relevance in comparison to cellular and molecular signatures observed in vivo.

Organ-on-chip (OoC) is a transdisciplinary technology that has emerged as a result of advances in several fields, such as microfluidics, cell biology, bio-engineering, physiology, and computational biology. OoC is a microfluidic device the size of an AA-battery and consisting of two (less than 1 mm wide) linear hollow channels separated by thin porous membrane (Fig. 23.1). On one side of the membrane, different organ-specific cells can be cultured, while the endothelium of the same organ can be cultured on the other side. The culture medium flowing through the vascular channel mimics the vascular perfusion and other cells, such as immune cells, connective tissue cells, and so on, can also be incorporated into the system. These microfluidic devices are designed to mimic and recapitulate the key physiology and functions of a single organ or a network of organs.

In the following sections, we discuss the origins of the organ-on-chip research and the various aspects to be considered during the designing of a chip.

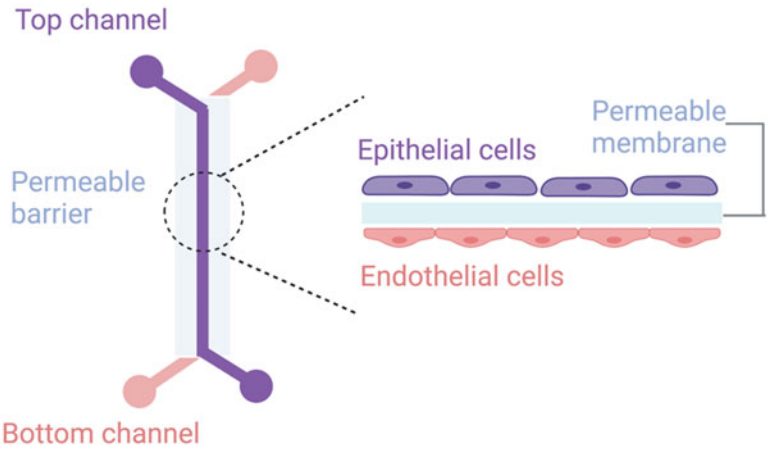

Fig. 23.1 The concept of organ-on-chip. The chip is made up of two channels, an upper layer with epithelial cells and a bottom layer consisting of endothelial cells. A permeable barrier/membrane separates these two layers. (Image created with BioRender.com)

23.1.1 History of OoC Research

Michael Schuler and Donald Ingber labs performed and pioneered the early OoC research. In 2004, "cell culture analogue" (CCA) system was used in conjunction with a pharmacokinetic model to predict human response in clinical trials [6]. The CCA was made up of a 1-in.^{2} silicon chip on which a microscale cell culture device was fabricated. This structure housed the mammalian cells that were cultured in interconnected chambers with recirculating tissue culture media which served as the blood substitute. The device could achieve physiological liquid-to-cell ratios, hydrodynamic shear stress, and real-time sensor integration to monitor and track parameters like oxygen. By 2009, advances in microfabrication and microfluidics led to the development of various organ chips, like blood vessels [7, 8], muscles [9], bones [10], airways [11], liver [12, 13], brain, gut, and kidney. On the other hand, integrated microsystems, incorporating multiple tissues to capture the complex functions of living organs, were still being developed.

A study published in 2010 described the re-creation of a microsystem capable of capturing the structural, functional, and mechanical properties of the human alveolar-capillary interface [14] (Fig. 23.2). This device consisted of two closely apposed microchannels that housed human alveolar epithelial cells and human pulmonary microvascular endothelial cells on the opposite sides. These channels were separated by a porous, flexible polydimethylsiloxane (PDMS) membrane coated with extracellular matrix. The cells were grown to confluence inside these chambers, before air was introduced into the epithelial compartment. This strategy created an air–liquid interface. Furthermore, two lateral microchambers were incorporated, and application of vacuum to these chambers caused the PDMS membrane, and the tissues adhered to it, to stretch. Following the release of vacuum,

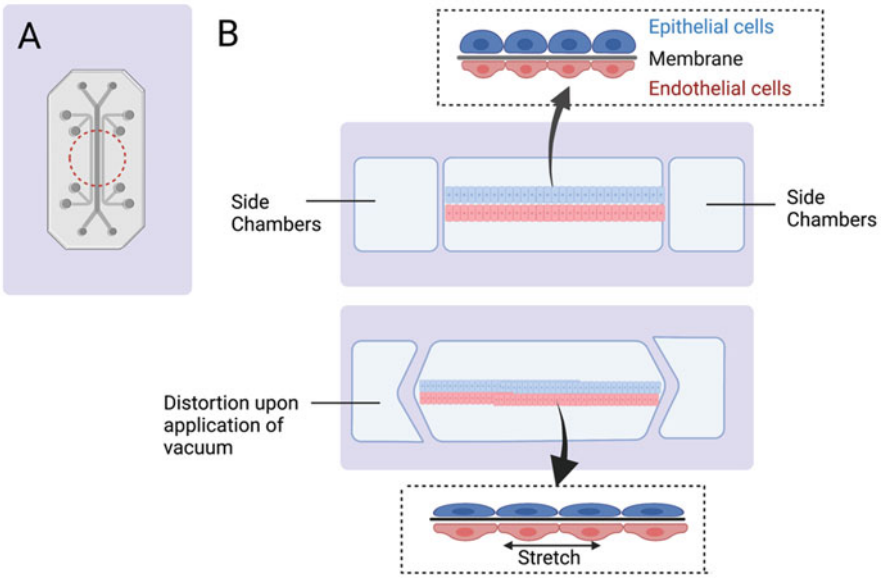

Fig. 23.2 Breathable lung-on-a-chip model. (**a**) Schematic of the lung-on-a-chip with dotted red circle indicating region magnified in the inset (**b**). (**b**) The chip is made up of two layers of epithelial cells and endothelial cells separated by membrane. This chamber is surrounded by two side chambers, which undergo distortion upon application of vacuum, causing the middle chamber to stretch. The use of such periodic distortion mimics the stretching-relaxation phenomenon seen during physiological breathing. (Image created with BioRender.com)

PDMS membrane elastically recoiled and caused the cells to relax back to their original state. This periodic stretching and relaxation movement of the tissues simulated physiological breathing in vivo. This device was capable of replicating the organ-level responses towards bacteria and inflammatory cytokines released into the alveolar space.

23.1.2 Design Considerations

The OoC system must support a cellular co-culture for an extended period of time, and mimic the spatiotemporal dynamics of cellular behaviours. The following are the critical considerations during the design of an OoC:

1. Maintaining a supply of nutrients and waste removal in the system.
2. Material for fabrication of OoC device.
3. Mimicking cellular and tissue microarchitecture.
4. Allometric scaling for maintaining cell ratios.
5. Long-term performance.

23.1.2.1 Flow Parameters

The in vivo cellular system involves the interaction of cells/tissues with blood and circulating substances. To replicate this system, a fluidic circuit with a steady flow of circulating media flow must be built. Furthermore, because the microfluidic channels contain such small volumes of media, they are prone to generating bubbles, which can jeopardise their functionality. One of the crucial factors of OoC is the perfusion, or flow of the culture medium or blood. Perfusion helps to maintain a constant nutrient supply and remove cellular waste that could lead to cell toxicity. The media flow is also designed to mimic the flow-induced shear stress [15] and delivery of dissolved oxygen to cells. However, dissolved oxygen can only meet 2% of the cellular oxygen demand [16]. Since increasing the flow rate to improve oxygen solubility can dilute metabolites and cause cellular injury, studies have used synthetic oxygen carriers to provide a more effective means of delivering oxygen to cultured cells [17].

23.1.2.2 Materials for OoC Device Fabrication

The material used to manufacture OoC devices as well as the lack of standards to regulate their mass production continue to be a barrier. Due to several advantages, such as high elasticity, gas permeability, optical clarity (which supports live imaging), and biocompatibility, PDMS is currently the most widely used material for microfabrication using the soft lithography technique. However, because PDMS absorbs small biomolecules, organic compounds, and drugs, it has some drawbacks that could affect the pharmacokinetic-pharmacodynamic (PKPD) parameters [18]. To address the limitations posed by PDMS, the use of several other materials, such as polystyrene (PS) 89 and polymethylmethacrylate (PMMA), are currently being explored.

23.1.2.3 Recapitulating Cellular and Tissue Architecture

Cellular organisation and connection between different organ modules in vivo contribute towards its functionality. In OoC, the system can be microfabricated such that cells and tissues can be organised into appropriate architecture. Microfluidics and perfusion aid in the integration of the cells and tissues into a single circuit connected via fluid exchange. Various approaches have been used to connect the cells and tissues within a system. The conventional approach is to use a standard cell culture medium. However, as cell metabolism varies from organ-to-organ and with respect to cellular maturity, this design requires more careful consideration.

23.1.2.4 Allometry

While there has been an increase in the number of publications on multi-cellular and multi-organ chips, the number or ratios of various cell types used in these systems (allometry) remain a point of ongoing research. It is critical to maintain relevant cell surface areas and cellular ratios while developing physiologically relevant models. Different models for allometric scaling have been proposed so that similar relationships can be replicated in a downscaled in vitro system. To accurately

recreate an in vivo organ system, an OoC must incorporate allometry. There are currently two types of scaling models in use: metabolic scaling models and cell number scaling models.

A metabolic scaling model, for example, was used to create liver OoCs. The liver is involved in nutrient uptake and distribution, while vascular tissues relay signals to distant organs. Biotransformation, the process by which organic compounds are transformed from one form to another, depends on the metabolic efficiency of liver cells; however, the distribution process is surface-mediated. In one study, hepatocytes involved in biotransformation were scaled based on basal metabolism, while endothelial cells involved in transport and distribution of various factors were scaled using the surface area of the vascular system as a reference point. The liver accounts for 27% of the total basal metabolic rate of a human. The study calculated the basic metabolic rate (BMR) per hepatocyte to be 119 pW based for a population of two billion hepatocytes. The researchers then calculated the equivalent BMR of the in vitro chamber of liver as 30 mW, as well as the number of human hepatocytes required for downscaled BMR contribution [19]. Similarly, cell number is also an important parameter to determine the rates of filtration and absorption. The liver and vascular endothelial tissue make up 6.2% and 2.6% of the total human body mass, respectively. Based on these rations, the relative numbers of endothelial cells and hepatocytes were calculated in the study.

23.1.2.5 Maintaining Consistent Performance for OoC Devices over Extended Periods of Time

It is also critical to ensure cell viability and functionality throughout the life-span of an OoC model to achieve reproducible results in vitro. This includes tracking, monitoring, and measuring various parameters of the devices, such as fluid flow, temperature, pH, oxygen, and CO_2. Aside from that, the functional characterisation of the model must be carried out. For this, assays and sensors are used to monitor cellular differentiation, nutrient and metabolite status, motility and migration, immune competence, and other factors.

23.2 Disease Modelling on a Chip

Due to the limitations in recapitulating the human pathophysiology in animal models, research groups across the world have been designing microfluidic devices based on human cells to understand various human diseases. In this section, we discuss five broad categories of disorders to understand how organ-on-chips are being developed to model diseases and discover novel drug targets.

23.2.1 Inflammatory Disorders

Our immune system protects us from invading microbes and foreign agents. The immune response can be acute or chronic, with acute inflammation referring to early

protection, and chronic inflammation being more complex and lasting longer. Inflammatory processes have been linked to disease progression in a variety of pathological conditions, including heart disease [20], cancer [21], chronic pulmonary disease [22], stroke [23], Alzheimer's disease [24], diabetes [25], and others. Inflammatory bowel disease (IBD), which includes ulcerative colitis and Crohn's disease, are chronic inflammatory conditions of the gastrointestinal tract with many of them being linked to inflammatory liver diseases. Their pathogenesis and interconnection are not fully understood. The complexity of animal models makes it difficult to control experimental factors to understand the underlying mechanisms, and the current in vitro models do not accurately represent the in vivo cellular diversity and physiology. As a result, the lack of effective treatment for inflammatory bowel disease necessitates the development of better models that can also allow for high-throughput screening of molecules.

A gut-on-a-chip model has now been developed using co-cultures of microvascular endothelial cells and intestinal myofibroblasts [26]. The gut-on-a-chip also addresses the major limitation of human intestinal organoids, which have an enclosed and spherical lumen that limits the drug exposure to the lumen [27]. A recent study reported the development of a gut-on-a-chip model using primary material derived from the patients. In this "membrane-free-3-lane OrganoPlate platform," macrophages derived from monocytes and human intestinal organoids from various donors were combined. This platform is made of glass and offers a higher throughput option than PDMS. The authors demonstrated that the transcriptome of this gut-on-a-chip mimicked that of an adult human colon in vivo. IBD was then induced by increasing the cytokine production by the macrophages in this system with lipopolysaccharide and interferons. Anti-inflammatory compounds were used in proof-of-concept experiments to prevent inflammatory phenotype in a dose-dependent manner. The authors proposed the use of this technology for large-scale screening to look for new IBD treatments.

In another study, researchers created a co-culture of ulcerative colitis gut MPS with a liver MPS (using healthy human hepatocytes and Kupffer cells) along with circulating regulatory T cells and inflammatory Th17 cells to model gut-liver-immune axis [28]. They discovered that depending on the involvement of effector CD4T cells, microbiome-derived short-chain fatty acids (SCFAs) can either improve or worsen ulcerative colitis severity. To better understand the link between ulcerative colitis and liver function, the researchers also looked at gene transcription, cytokines, and metabolomic changes in MPS tissues.

23.2.2 Cardiovascular Diseases

Cardiovascular diseases is one of the leading causes of death worldwide [29]. Atherosclerosis, a chronic condition in which the vasculature architecture is gradually remodelled, is one of the primary diseases of vasculature. This is a complex disease involving the interaction of several cell types, including endothelial cells (ECs) (which eventually become dysfunctional), leukocytes, and macrophages (which

trigger inflammation), and smooth muscle cells (which trigger apoptosis) [30]. Atherosclerosis is associated with the formation of plaques in the arteries which can often rupture, resulting in arterial thrombosis or the formation of blood clots and conditions, such as myocardial infarction (MI) and ischaemia. MI causes myocardial remodelling, the mechanisms of which still are unclear and increase the re-hospitalisation rates. Cardiac remodelling is characterised by an increase in extracellular matrix deposition, which leads to cardiac fibrosis, chamber dilation (dilated cardiomyopathy), and cardiomyocytehypertrophy [31].

Organ-on-chip devices offer a high level of control over the cell-culture environment including the topology extracellular matrix (ECM), sensors for monitoring various parameters, microfluidic channels for spatial and temporal flow control. The advancements in microelectronics have provided methods for defining the dimensions of the microfluidic channels ranging from a few micrometres to a few millimetres. To mimic the in vivo blood vessels, the microfluidic channels can be coated with human endothelial cells, which can form artificial intima (inner-most) layers of vessels, allowing blood, plasma, platelets, etc. to be perfused. Creating perfusable microvascular networks, on the other hand, has been a challenge. The stiffness of microvascular networks engineered with solid polymeric materials is much higher than that of the tissues that surround blood vessels, which can lead to endothelial dysfunction [32, 33]. Although hydrogel-based microvascular networks have a more physiologically relevant stiffness, they can be unstable in terms of vascular size, length, and geometry which could contribute towards uncontrolled flow patterns [34, 35]. A recent study developed a microvascular-sized microfluidic device using an agarose–gelatin interpenetrating polymer network (IPN) hydrogel. This device could measure the in vivo diameter of venules, shear stress, blood vessel stiffness, and could be maintained for over a month under laminar flow conditions [36].

23.2.2.1 Atherosclerosis

Several studies have attempted to model atherosclerosis on a chip. A square channel with an embedded artificial plaque was used in a few studies [37, 38]. Another study used 3D printing of computed tomography angiography (CTA) data to create a 3D vessel with a diameter of around 400 μm [39]. Human umbilical vein endothelial cells (HUVECs) were then coated on the microfluidic chip walls. The devices with healthy geometries did not show signs of thrombosis after being perfused with human whole blood for 15 min, whereas the devices with stenotic (or narrowed) geometries induced thrombosis downstream of the stenotic area. Many of these models, however, lack ECM, and addition of monocytes and macrophages to the system would help to create more physiologically relevant atherosclerosis models.

23.2.2.2 Thrombosis

Thrombosis can be caused by abnormalities in the vessel wall, blood components, or turbulence in the fluid [40, 41]. The flow parameters as well as its interaction with vessel walls, cannot be captured using the static 2D in vitro models. Many thrombosis organ-on-chips have a tubular or rectangular mould that is further covered by

extracellular matrix. The endothelial cells form a monolayer which mimics the geometry and function observed in vivo. Endothelial cells have anti-coagulant and anti-inflammatory properties in a healthy state, allowing blood flow and preventing clot formation [42]. Endothelial damage, on the other hand, may trigger the thrombus formation.

Several organ-on-chip studies have tried to figure out the triggers for clot formation. One study engineered microvascular networks by seeding HUVECs into collagen-coated microfluidic circuits. When this system was perfused with human blood, platelets selectively adhered to the sites of damage [43]. Recently, a "vessel chip" has been developed to monitor various parameters during thrombosis, such as endothelial activation, platelet adhesion, platelet aggregation, fibrin clot formation, and others [44]. The researchers also perfused the whole blood to investigate drugs that could promote clot formation.

23.2.3 Neurological Diseases

While the global incidence of neurological diseases is on the rise, the 2018 Tufts Centre for the Study of Drug Development Impact report states that drugs for the central nervous system (CNS) take 20% longer to develop and 38% longer to approve [45]. Additionally, CNS drugs have a higher risk of failure during the late stages of clinical trials. The enormous differences in structural and functional complexities between animal and human brains may contribute to these translational challenges. Because of the high failure rate, the top ten pharmaceutical companies in the world have scaled back their drug development programmes in neurosciences [46]. This emphasises the importance of developing model systems based on human biology in order to better understand human diseases.

23.2.3.1 Alzheimer's Disease

Over three decades of research has revealed the molecular and cellular events that underpin Alzheimer's disease, including accumulation of amyloid-β (Aβ) and the hyperphosphorylated, microtubule-associated protein, Tau. The exact mechanisms underlying its pathophysiology, however, remain unknown. Tau pathology is known to spread via trans-synaptic transfer of Tau between neurons, and the Tau species involved in this propagation between neurons has been studied recently using a microfluidic neuron culture platform [47]. This platform consisted of three distinct chambers—two neuron sets are plated on chambers 1 and 2, where axons from the first chamber extend to the second chamber, and the neurons from the second chamber extend into third chamber. The study of synaptic connections in the second chamber revealed that a rare high-molecular weight Tau species was uniquely capable of propagating between neurons. Another study found that certain Tau antibodies could block the Tau protein uptake, aggregation, and spreading [48], implying that Tau-targeted therapeutics could be developed. By regulating the micro-environment in a bio-mimetic microfluidic system, researchers investigated the neurotoxicity of amyloid beta proteins [49]. The authors were able to

demonstrate that amyloid beta exposure causes atrophy in neurons cultured in continuous flow using spatial gradients of diffusible amyloid beta proteins.

A three-dimensional (3D) human Alzheimer's disease tri-culture model was recently created in a 3D microfluidic platform using neurons, astrocytes, and microglia [50]. The key features of the disease, such as beta-amyloid aggregation, phosphorylated Tau accumulation, and neuroinflammation were all well-replicated in this model. This model could in fact aid in the development of new models to understand neural–glial interactions and drug discovery in Alzheimer's disease.

23.2.3.2 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disease that affects more than six million people worldwide. It is characterised by the loss of dopaminergic neurons in the substantia nigra, a basal ganglia structure in the midbrain region, and the aggregation of intracellular proteins like alpha-synuclein (α-Syn), a presynaptic protein. Small oligomers of α-Syn aggregate into fibrils, which then aggregate and accumulate in intracytoplasmic and intraneuritic deposits known as Lewy bodies and Lewy neurites. Many microphysiological systems models have recently been used to investigate the mechanisms of α-Syn aggregation and trafficking. Researchers used iPSC cells from healthy people and people with PD (who had α-Syn duplication) and differentiated them into neurons in microfluidic chambers to investigate the role of α-Syn oligomers in neurite pathology [51]. They discovered that an increase in α-Syn oligomers can compromise the axon integrity and impair mitochondrial transport.

A novel microfluidic cell co-culture model was used in another study to understand how different cellular populations interact during PD [52]. To investigate the release and spread of the protein, they grew naive H4 human neuroglioma cells that expressed α-Syn tagged to green fluorescent protein (GFP). When H4 cells were grown in the presence of N9 microglial cells derived from mouse brain in another set of experiments, they saw an increase in the levels of reactive oxygen species. This indicated the potential of this system to understand the interplay between different cell populations during disease manifestation. Another 3D microfluidic platform was used to grow PD patient-specific neurons derived from induced pluripotent stem cells [53]. In the absence of external stress, these neurons displayed various hallmarks of PD, including decreased dopaminergic differentiation and branching complexity, altered mitochondrial morphology, and increased cell death. They identified that the phenotypes were greatly influenced by the genetic background of the PD patient, rather than the α-Syn-specific mutation. This model was also shown to be suitable for high-content imaging and drug testing.

23.2.3.3 Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease characterised by demyelination; however, the impact of demyelination on neurons and axons is not clearly understood. In vitro assays for the generation of myelinating oligodendrocytes from mouse embryonic stem cells have been developed, as well as a myelin formation assay using embryonic stem cell-derived neurons in microfluidic devices [54]. In

another study, researchers developed a compartmentalised cell culture device involving the co-culture of neurons and oligodendrocyte precursor cells [55], wherein myelination could be induced by exposing the axonal compartment to lysolecithin. This system was used to discover a process in which mitochondria move from the neuronal cell body to the demyelinated neuron, and that improving this process helped to protect demyelinated axons from degeneration. The researchers proposed that promoting mitochondrial axonal response to demyelination could be a critical step in investigating therapeutic studies for demyelination-related disorders, like MS.

Another study used a microfluidic biochip to co-culture three-dimensional motor neuron-and Schwann cell (MN-SC) [56]. While the chip allowed SCs to interact with the MN axons, any direct contact was prevented between SCs and the cell bodies of MNs. Light stimulation accelerated the initiation of myelination in MNs transfected with a light-sensitive channel rhodopsin gene. This suggests that this platform could be used to study and understand neuronal regrowth and myelination.

23.2.4 Cancer

Currently, preclinical development of cancer therapeutics is based on results from animal models; however, these models are known to poorly predict the human pathophysiology. According to studies, the approval rate for oncology drugs is lower than for other diseases. While one out of every eight drugs that enter the clinical development pipeline is approved by the FDA, one out of every fifteen drugs in oncology is approved [57, 58]. Furthermore, oncology drugs have the lowest approval probability at 6.7% [58]. Here, we highlight some of the studies that explored the use of microfluidic systems to understand specific steps of the cancer cascade, such as cancer microenvironment, angiogenesis, epithelial-to-mesenchymal transition (EMT), tumour invasion, and metastasis.

During the progression of cancer, there are frequent changes in the 3D microenvironment and composition of the extracellular matrix [59]. Traditionally, 2D cell cultures do not account for the influence of stromal cells or the role of ECM molecules in the modulation of cancer cells. One study used a tri-culture of three different cell types, including breast cancer cells, stromal cells, and monocytes and performed gene expression analysis to understand how tumour and stromal cells respond to various microenvironments [60]. Another interesting study also found that conditioned medium from a healthy alveolar epithelium could promote the proliferation of cancer cells [61]. In addition, they demonstrated that recapitulation of physiological breathing motions in the chip via application of cyclical strain on the alveolar-capillary interface of the chip could suppress cancer growth and invasion.

Several studies have now shown that angiogenesis is one of the critical steps towards unrestricted growth of tumours [62]. Studies have also developed microfluidic angiogenesis models, which simulate vessel sprouting and formation using engineered microvessels. Use of engineered vessels using preformed artificial

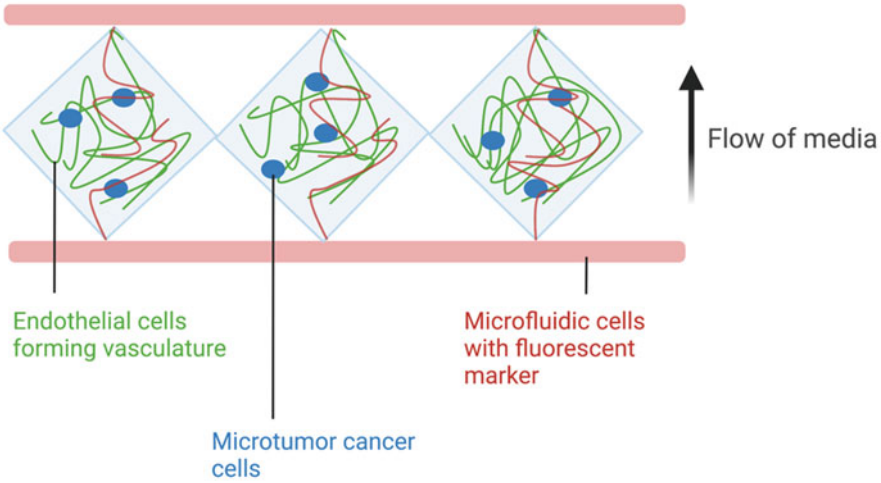

Fig. 23.3 Vascular network formation in individual tissue units of microfluidic tumour model. Microvascular networks were formed in diamond-shaped cell chambers seeded with human endothelial colony-forming cell-derived endothelial cells (in green). Seeding the three tissue chambers with HCT116 colorectal cancer cells resulted in the formation of vascularised microtumours (in blue). The perfusion of medium through the system was observed using rhodomine B-dextran (in red). (Image created with BioRender.com)

vessels that were completely encapsulated in a 3D extracellular matrix [63] exhibited typical features of in vivo angiogenesis, such as leading cell filopodia-like projections, apicobasal polarity of stalk cells, and branches connecting to parent cells and lumen. This model was also used to investigate targets that could both inhibit and stimulate angiogenesis. One study demonstrated the use of microfluidic system to create vascularised micro-organs and microtumours for in vitro drug screening [64] (Fig. 23.3). The platform was the size of a standard 96-well plate and consisted of twelve tissue units with fluid flow driven by hydrostatic pressure. The system was designed such that each unit could be fed a different drug or drug dose. Furthermore, the tissues could be easily extracted for gene expression analysis. The utility of this system was demonstrated using colon tumour cells.

Epithelial-mesenchymal transition (EMT) is a complex developmental programme, in which cancerous cells suppress epithelial features and transition to a more mesenchymal cell fate. This transition allows the cells to acquire motility and ability to migrate which is a key step during metastasis. Transwell systems, a type of permeable support that fits into multi-well cell culture plates, have traditionally been used to study anti-cancer drug resistance, angiogenesis, EMT, and other topics. However, they provide a non-physiological distance between the two cellular compartments, resulting in the dilution of cellular factors. In one study, indirect co-culture microfluidic chip was designed wherein the tumour cell and stromal cells were not in direct contact but soluble factors could be transported across a 1000 μm medium channel allowing the development of local concentration gradients. When

the tumour spheroids and CCD-18Co fibroblasts were co-cultured, the stomal cells were able to induce EMT in the cancerous cells [65]. Another study investigated the role of fluidic forces as modulators of EMT and metastasis [66]. The report showed that continuous flow could induce post-translational upregulation of epidermal growth factor receptor (EGFR) activation and expression, which is linked to poor prognosis in ovarian cancer. Furthermore, flow-induced decrease in E-Cadherin and increase in vimentin expression, both of which are indicators of the EMT phenotype.

One of the crucial steps during metastasis is the invasion of tumour cells into the bloodstream [67]. In a study, the authors developed a microfluidic-based assay to quantify endothelial barrier function by simulating tumour vasculature interface [68]. Using this system, they demonstrated that macrophages caused endothelial barrier impairment and an increase in intravasation rates by secretion of tumour necrosis factor alpha. They also discovered that barrier dysfunction was linked to increased tumour-endothelial (TC-EC) signalling. Another group developed a microfluidic system that combined tumour and endothelial cell co-culture in a 3D environment [69]. The system included a vascular compartment with a network of vessels cultured with endothelial cells that formed a complete lumen as well as a tumour compartment with 3D solid tumours. The authors showed that exposure to tumour cell conditioned media (TCM) or tumour cells themselves increased the permeability of endothelial cells.

23.3 Pharmacokinetic Pharmacodynamic Analysis Using Organ-on-Chips

One of the key challenges during drug development is the need to develop relevant models that can predict the pharmacokinetic (PK) parameters, including how the drugs are absorbed, metabolised, distributed, transported, and excreted in the body (also referred to ADMET parameters). Pharmacodynamics, on the other hand, refers to how the drug acts at the target site and also attempts to quantify the relationship between the drug concentration and its efficacy and potency. A PK-PD model should be able to predict fairly efficiently whether a particular dose regimen of a drug can achieve clinically relevant plasma levels in vivo. In this section, we discuss the relevance of specific PK-PD parameters, and the use of microfluidic chips to infer physiologically relevant drug kinetics and dynamics.

23.3.1 Relevant Pharmacokinetic Parameters

Various methods are used in drug development to estimate the pharmacokinetic parameters listed below:

- Maximum concentration (C_{max})
- Maximum time to reach maximum concentration (t_{max})
- Area under the curve (AUC)

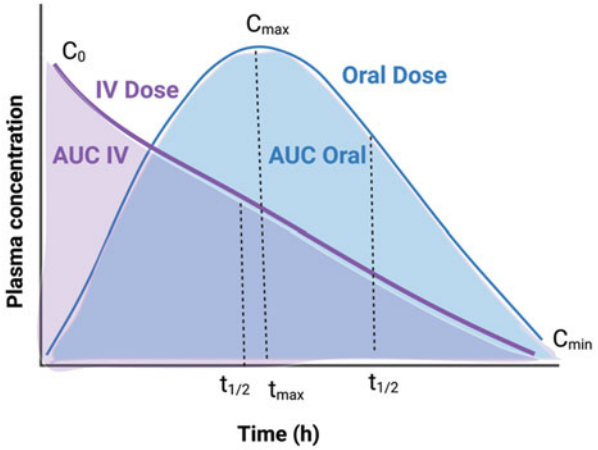

Fig. 23.4 Concentration versus time graph for pharmacokinetics. The graph depicts calculation of key PK parameters from drug exposure curves, including the area under the curve (AUC) that reflects the time-dependent exposure of the drug over time in vivo, maximum concentration for orally administered drug (C_{max}), and minimum concentration of the drug (C_{min}), time for the plasma concentration to decrease by 50% ($t_{1/2}$), and the time required to reach C_{max} (t_{max}) after oral administration of the drug. (Image created with BioRender.com)

- Bioavailability (F)
- Clearance (CL)
- Volume of distribution (V_d)
- Drug half-life ($t_{1/2}$)
- Elimination rate constant (k)

For determining these parameters in vivo, a single bolus of drug is administered either intravenously or orally and the systemic blood or plasma concentration is measured as a function of time. These concentration measurements, when plotted against time can reveal the systemic exposure of the drug as a function of time (Fig. 23.4). The peak values of the curve are used to calculate C_{max} and t_{max}, as well as area under the curve (AUC). Bioavailability (F) refers to the fraction of the drug that reaches the systemic circulation post oral administration. Various factors may play a role in determining this parameter, including absorption across the small intestine, intestinal epithelial permeability, metabolism, active transport via efflux/influx transporters, etc. In addition to the intestinal absorption and metabolism, hepatic clearance and the route of administration can also influence bioavailability.

The term "clearance" (CL) refers to the process of removing a drug from systemic circulation in the body over a period of time. Bile secretion in the liver plays a major role in drug metabolism. Similarly, kidney is another critical organ due to elimination processes occurring during the proximal tubular transport and/or glomerular filtration. Drug clearance is also affected by blood flow rates, plasma protein binding, activity of various transporters, amongst other factors.

Volume of distribution is the volume into which the drug distributes in the blood compartment. This parameter varies as a function of time, tissue penetration, and drug binding. It also varies depending on whether the drug exposure is in a steady state, such as constant IV infusion, a peak state (C_{max}), or an elimination phase. The $t_{1/2}$ is the amount of time it takes for the drug concentration to drop by 50% after oral administration. This parameter is calculated using a concentration versus time graph in which the concentration rises until it reaches C_{max} and then gradually tapers off due to liver and kidney elimination processes.

23.3.2 Relevant Pharmacodynamic (PD) Parameters

To determine the drug-target binding and dose-effect relationships, scientists have traditionally used cellular assays such as in vivo or in vitro drug titration assays. However, because the in vivo drug efficacy is affected by a variety of factors, such as blood flow and binding process over time, these assays are frequently insufficient to predict it. Animal models and in vitro assays have been used to determine the PK-PD parameters, but both have significant drawbacks. Differences in body size, circulatory flow rates, liver and gut enzymes, and transporters, all of which can have a significant impact on ADMET measures, make animal models poor predictors of this [3, 70–72].

To evaluate the drug absorption and efflux process in vitro, Caco2 intestinal epithelial cells (originally isolated from a human colorectal adenocarcinoma) or the Madin-Darby canine kidney (MDCK from kidney tubule of an adult cocker spaniel dog) epithelial cell lines have been routinely used. However, the translatability of these models is debatable. The permeability of the small intestine, where drug absorption occurs; mucus layer; and fluid shear stress in vivo can all affect drug absorption and bioavailability. A static 2D culture captures none of these variations, reducing its physiological relevance.

23.3.3 PK-PD Modelling Using Microfluidic Organ-on-Chip Models

Several studies are currently attempting to develop a microfluidic system using human cells to generate a physiologically relevant model for determining the PK-PD measures. The authors of a study created a “heart-on-a-chip” that was connected to a “liver-on-a-chip” to evaluate temporal pharmacokinetic/pharmacodynamic (PKPD) relationship for an anti-histamine drug, terfenadine [73]. This platform used a pump-less system, which resulted in a bubble-free, low volume system that could detect low levels of metabolites. This system was used to calculate the PK-PD relationship for terfenadine, an antihistamine, and the data was then used to build a mathematical model to predict PK-PD parameters. The researchers also compared their findings to in vivo results in preclinical animals. Finally, they tested a previously discovered proprietary AstraZeneca small molecule in this heart–liver

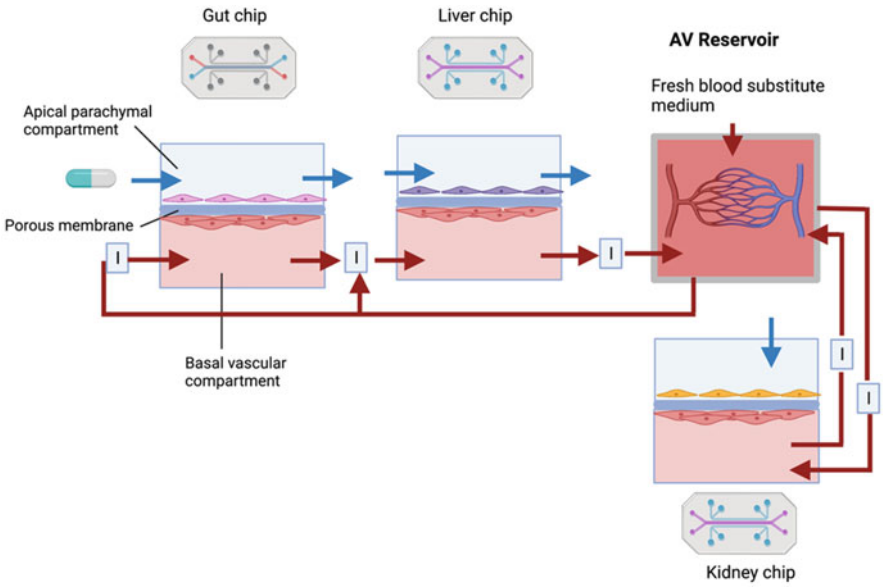

Fig. 23.5 Fluidically coupled multi-organ-on-a-chip. The diagram shows gut, liver, and kidney chips that consist of an upper parenchymal and lower basal vascular compartment separated by a porous membrane. The chips are connected fluidically to one another and to the AV reservoir, which represents systemic circulation and can be used to measure blood and plasma concentrations. The path and flow of medium are indicated by red arrows and "I" indicates the sites where the fluid transfer was completed using an automated liquid-handling instrument. (Image created with BioRender.com)

system to see if the system could correctly predict the cardiotoxic response to this metabolite.

In 2012, the Defense Advanced Research Projects Agency (DARPA), a research and development arm of the US Department of Defense, partnered with Harvard University's Wyss Institute for Biologically Inspired Engineering to develop a functional human Body-on-Chips platform in order to speed up drug target development. Two papers published recently as part of this project describe the successful development of a "body-on-chip," which, in combination with computational modelling, could predict human drug PK-PD responses [74]. This platform can culture up to ten organ chips and transfer fluids between them in a sequential manner via vascular channels lined with endothelium to mimic in vivo blood flow. The medium flows directly from one "organ chip" to the next passing through an endothelial tissue barrier. One of the major limitations in existing microfluidic platforms is that the medium flows directly from one "organ chip" to the other without passing through an endothelial tissue barrier. This reduces the physiological relevance of the measured pharmacokinetic parameters. To overcome this limitation, the researchers devised a system in which gut-on-a-chip, liver-on-a-chip, and kidney-on-a-chip were fluidically connected via vascular endothelium-lined channels

(Fig. 23.5). This is a critical design consideration since drugs usually pass through an endothelium-parenchymal tissue interface as they move from one organ to the next, and endothelium also plays a role in ADME and PK properties. The authors also included an arteriovenous reservoir in the fluid path for drug mixing. This reservoir mimics the systemic circulation in vivo and can be used for measuring the drug concentrations in blood and plasma—a critical parameter during PK calculations.

The authors also developed an "Interrogator" instrument that used liquid-handling robotics to automate the fluid linking, medium addition, sample collection, and in situ microscopic imaging [75]. This instrument can keep up to eight organ chips alive and functioning (intestine, liver, kidney, heart, lung, skin, blood-brain barrier (BBB), and brain) for up to 3 weeks. This device also enabled automated non-invasive imaging of compartments without disrupting fluidic coupling, which could compromise the viability and functionality of the cells in the system.

The gut chip was lined with Caco-2 intestinal epithelial cells which show intestinal villi with all four epithelial cell lineages of the small intestine, barrier function, drug-metabolising cytochrome P450 activity, and mucus formation on the apical side. This cell line could also be replaced by primary intestinal cells isolated from patients. The PDMS gut chip was pre-coated with Matrigel and collagen type-I before being seeded with Caco-2 BBe cells in the upper channel and umbilical cardiovascular endothelial cells in the lower channel.

For creating liver chips, human primary liver sinusoidal microvascular endothelial cells were plated on the lower side of PDMS membrane along with human primary hepatocytes on the upper side. Kidney chips were generated by seeding primary human glomerular microvascular endothelial cells and primary human renal proximal tubule epithelial cells on the lower and upper sides of the chips containing PTE membranes pre-coated with Collagen IV and laminin. Using the experimental data obtained from this model, the authors then built an in silico PK-PD model and performed an in vitro to in vivo translation. Furthermore, they were able to use this system to recapitulate the organ toxicity of a well-known drug Cisplatin, proving the validity of this model. However, some other organs that can influence PK parameters, such as fat tissues that can absorb drugs, were missing from this system. This was compensated in this system during in silico modelling, where fat tissues were virtually added by altering the absorptive properties of PDMS material of the organ chip or altering the dimensions of the organ-chip such that it predicts the in vivo state. While this was done to mimic the PK properties of nicotine and cisplatin, further optimisation of the absorptive properties of PDMS would be required for drugs with different properties. The glomerular function, which significantly contributes to the urinary clearance and was not recapitulated in the microfluidic system, was another aspect that was compensated during the in silico.

23.4 Designing Various Organ Chips

Apart from modelling diseases, research groups are designing organs chips to mimic specific organs for understanding their function and pathology. In this section, we discuss the advances in the development of fiver organ chips: heart, liver, lung, kidney, and brain chip and the development of therapeutics to address organ dysfunctions.

23.4.1 Heart-on-a-Chip

Heart disease is one of the leading causes of death worldwide, and predicting the drug cardiac toxicity remains a challenge during the drug development process. A study that aimed to understand the withdrawal of drugs from the market due to adverse events found that hepatic, cardiac, and nervous system toxicity was responsible for over 60% of drug withdrawals [76]. All of these drug candidates passed the preclinical safety testing on animal models, indicating the need for more precise and relevant models for preclinical testing. For instance, the cardiac electrophysiology of animals such as mice and humans differ in several ways. While mice can only increase their cardiac output by a small amount, humans can increase their cardiac output by a factor of 5–6 [77]. In addition, mechanisms of arrhythmia have also been shown to differ between humans and mice. Several studies have used iPSC-induced cardiomyocytes (CMs) for drug safety evaluations in the last decade [78]. However, one of the drawbacks of single cells is their inability to predict the kinetics and response of myocardial tissue, which includes stromal cells, vascular cells, and other cell types. Thus, an ideal system would consist of biomimetic heart-like structures that included various cardiac lineages for assessing the physiological function of the heart. In order to study the physiology and mechanical properties of cardiac tissue, it is also necessary to mimic the cardiac microenvironment. Cardiomyocytes (CMs) are arranged in layered sheets that are connected intracellularly via cell-cell junctions, including adherens and desmosomes, in the myocardium, or muscle tissue of the heart. The cytoskeletal filaments actin and desmin are connected to these cell–cell junctions. Disruptions in these cell–cell junctions can cause a variety of cardiovascular diseases [79].

Recent research has resulted in the development of a Micro Electro Mechanical System (MEMS), which is a microdevice that can mimic the heart's pump function and has a sensitive bioassay system for cardiac toxicity and drug discovery tests. One study, for example, designed a micro-spherical heart-like pump that was powered by spontaneously contracting cardiomyocyte sheets [80]. A fabricated hollow elastomeric sphere with a diameter of 5 mm having inlet and outlet ports was wrapped around the beating cardiomyocyte sheet that exhibits large contractile forces. The spontaneous beating of CMs caused fluid oscillations in the capillary connected to the hollow sphere, which were confirmed to work continuously for 5 days.

In a recent study, researchers combined two technologies to create a heart-on-a-chip microdevice: MEMS-based organ-on-a-chip technology and human iPSC

technology [81]. This system was also tested for its ability to respond to electrical stimulation and dose-dependent administration of ionotropic drugs. The researchers first induced various cardiovascular lineages before harvesting iPSC-derived cardiac microtissues. These microtissues were then incubated in a PDMS microfluidic chip containing four components: the microchannel, chamber, diaphragm, and push bar. To promote attachment to the tissue, the chip was coated with fibronectin in phosphate-buffered saline (PBS) overnight at 37 °C overnight. The system was able to measure various cardiac kinetics and physiological parameters, such as fluidic output, pressure, and force, and showed a pharmacological response to isoproterenol, a known β-adrenoceptor agonist. The electrical properties of cardiomyocytes are detected or determined using impedance. With the help of high-speed impedance detection technology, a study developed a heart-on-a-chip device for evaluating drug cardiac efficacy [82]. This device assessed drug cardiac toxicity in a high-throughput preclinical setting by measuring multiple parameters of cardiomyocyte beating status and electrical properties.

23.4.2 Liver-on-a-Chip

Preclinical testing in rodent and non-rodent models is required by almost all drug regulatory bodies around the world before moving forward with clinical trials. According to a study that looked at the concordance of toxicity in animal models and humans, non-rodents and rodents were predictive for only 63% and 43% of human toxicity [83] in 150 drugs. The positive predictive value of drug-induced liver toxicity (DILI) between animal studies and clinical data was found to be low in a non-clinical to clinical database created by an industry-wide association [84]. Thus, one of the major challenges in the drug discovery pipeline is predicting liver-induced drug toxicity.

A bioartificial liver was created in one study using a surface-engineered microfluidic chip with microtrenches that mimicked hepatic sinusoids [85]. These sinusoids are microvascular structures that are important sites for exchange between oxygen-rich blood from the hepatic artery and nutrient-rich blood from the portal vein. They play a role in in liver injury, inflammation, and fibrosis, among other things [86]. The microtrenches are coated with 3D heparin, and the primary hepatocytes that live inside them secrete high levels of albumin and urea. This system replicated the in vivo cell–cell interaction and continuous flow through microfluidic vessel, and could be maintained for more than 4 weeks. The cytotoxicity of common drugs like acetaminophen, chlorpromazine, and tacrine was assessed on days 1 and 7 to validate this system. The closely aligned values of inhibitory drug concentration IC_{50} (chemical concentration of the drug that inhibits 50% of the processes) with the Lethal Dose 50 LD_{50} (the dose of the drug that will kill 50% population of animals in single oral administration values), with the values observed in vivo, indicated the effectiveness of this system.

Another study demonstrated a high throughput liver-on-a-chip system, titled OrganoPlate Liver Tox, which includes iPSC-derived hepatocytes seeded on organ

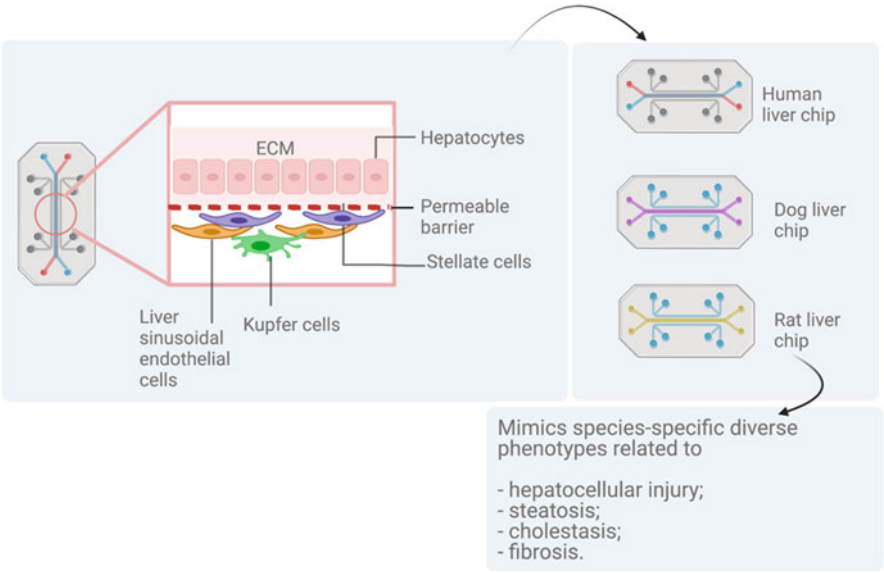

Fig. 23.6 Recapitulating the species-specific drug-induced liver toxicities. Schematic representation of a liver chip that mimics liver cytoarchitecture, including primary hepatocytes in the upper channel and non-parenchymal cells (LSECs, Kupffer, and stellate cells) on the lower vascular channel. This format was followed for building human, dog, and rat liver chips that were subsequently tested and validated to demonstrate species-specific drug-induced liver toxicities towards drugs. (Image created with BioRender.com)

channels as well as endothelial cells and differentiated THP-1 Kupffer cells in the perfusion channel to mimic the hepatic sinusoids [87]. While the hepatocyte to Kupffer cell ratio was close to that of the in vivo scenario, the hepatocyte to endothelial cell in the system was 50-fold higher than in vivo liver sinusoid. However, when the researchers tried to recapitulate the native situation with fewer endothelial cells, they were unable to achieve the 3D blood vessel-like structure. Over the course of 15 days, this model was evaluated for albumin urea, cell viability, and human cytochrome P450 3A4 (CYP3A4—the main enzyme involved in human drug metabolism). The ability to perform high-throughput screening, seeding, dosing collection, media replenishment, and assay reagent addition in an automated manner was a key feature of this system.

To better understand the species-specific differences in drug metabolism and toxicity, researchers created liver chips lined with rat, dog, or human hepatic cells [88] (Fig. 23.6). Primary rat, dog, or human hepatocytes were seeded in the upper parenchymal channel, while on the opposite side, relevant species-specific endothelial cells with or without Kupffer cells were seeded. They first examined the liver chips' physiological function and relevance, which were found to be comparable to in vivo levels. The researchers then used the system to predict hepatocellular injury, steatosis, cholestasis, and fibrosis in different species of drug-induced liver injury (DILI) responses, including hepatocellular injury, steatosis, cholestasis, and fibrosis.

They looked at the effects of Bosentan, an endothelin receptor antagonist that causes cholestasis in people, but not in rats or dogs. The results were correlated between the clinical response and the response in chips. To understand the diverse phenotypes of liver toxicity, they then incorporated nonparenchymal cells (NPC), hepatic stellate and Kupffer cells into the vascular channel to create a "quadruple-cellLiver-Chip model." This quadruple liver chip could detect diverse phenotypes of liver toxicity, including hepatocellular injury, steatosis, cholestasis, and fibrosis, indicating its utility in preclinical liver toxicity testing.

23.4.3 Lung-on-a-Chip

A seminal paper published by the Donald Ingber's group in 2010 described the development of a multifunctional microdevice that could recapitulate the structural, functional, and mechanical properties of the alveolar-capillary interface [14]. The device consisted of two microchannels separated by a flexible PDMS membrane with human alveolar epithelial cells and human pulmonary microvascular endothelial cells cultured on either side. After the cells were cultured, air was introduced into the compartment with epithelial cells creating an "air-liquid interface" that mimicked the alveolar space lining. The natural process of inspiration and expiration causes cyclical stretching of the alveolar epithelium, which was also recapitulated in this system. Two lateral microchannels were incorporated in the system. Application of vacuum and subsequent release-cased cyclical stretching and relaxation of the PDMS membrane and tissue layers adhered to it. This mimicked the alveolar-capillary interface distortions caused by breathing movement. The authors also demonstrated that this mechanical strain could significantly amplify the lung inflammatory response to toxic airborne particles (simulated using silica nanoparticles), which could not be detected using traditional static cultures.

However, the strain in this system depends on the viscoelastic properties and thickness of the PDMS membrane. Furthermore, the negative pressure must be precisely controlled. In another study, these limitations were addressed by using primary human pulmonary alveolar epithelial cells obtained from patients, and the 3D mechanical strain was delivered using a novel bioinspired actuation method, in which the lung-on-chip was connected to an external electro-pneumatic setup [89]. This setup enabled the control of the magnitude and frequency of the applied negative pressure, which was within the physiological strain experienced by the alveolar epithelium inside the lungs. The same team recently developed a second-generation lung-on-a-chip that addresses the limitations of the previous system [90]. They were able to create an array of alveoli with in vivo dimensions using this system. They replaced the PDMS membrane with a biodegradable and stretchable lung ECM, collagen, and elastin membrane. This membrane was created via drop-casting a collagen-elastin solution on to a gold mesh which spreads and forms a stable scaffold. This system could support about 40 alveoli while maintaining the air-blood barrier function in a reproducible manner.

In another study, which aimed to mimic the microstructure, ECM properties, and air-cell interface, the researchers developed a physiologically relevant human alveolar lung-on-a-chip model made of 3D porous hydrogel. This hydrogel was created using low-stiffness gelatinmethacryloyl (GelMA) with an inverse opal structure bonded to compartmentalised polydimethylsiloxane (PDMS) chip device [91]. The structure of GelMA had a high similarity to the human alveolar sacs as it possessed sac-like pores, interconnected windows between the sacs, and a similar in vivo stiffness. This system could also mimic the functions of primary human alveolar epithelial cells. Furthermore, it was used to study the pathological effects of cigarette smoking and SARS-CoV-2 infection.

23.4.4 Kidney-on-a-Chip

While animal models have been used to study whole-kidney responses, there are species-specific variations due to differences in circulation and blood flow properties, metabolising enzymes, and varying expression of transporter proteins [92, 93]. Every day human kidneys filter and reabsorb nearly 180 L of blood, making them more susceptible to drug/toxin damage or blood-borne diseases. The nephron is made up of glomerulus that purifies the blood via its ultra-filtration action and the proximal convoluted tubule lined with epithelial cells and aids in re-absorption of the solutes passing through the lumen fluid. While chronic kidney disease has many causes, such as metabolic disorders, hypertension, and autoimmunity, decreased glomerular filtration and functional glomeruli have been identified as a key early pathological biomarker [94]. The glomerular filtration is coordinated by the interaction of fenestrated endothelium and podocytes that is separated by a thin layer of glomerular basement membrane. One of the challenges in creating models for chronic kidney diseases lies in recapitulating the complex structure and function of the glomerular filtration barrier.

23.4.4.1 Glomerulus-on-a-Chip

In many of the glomerulus chips, the different cell types, including podocytes and glomerular endothelial cells, are separated by polydimethylsiloxane membrane which may not allow crosstalk between the glomerular cells. Researchers recently created a glomerulus-on-a-chip with podocytes and human glomerular endothelial cells (hGEC) seeded on Organoplates, a microfluidic 3D tissue culture plate that can support 96 tissue models on a single plate [95]. Because there was no artificial membrane separating the two monolayers in this study, cells could be cultured and maintained for longer periods of time. Furthermore, the interaction of the glomerular cells resulted in the formation of an extracellular matrix layer made up of collagen IV trimer and laminin, both of which are components of the glomerular basement membrane in vivo. Serum from people with a variety of glomerular diseases, including nephropathy, was used to test the chip's validity. They also created a chip with podocytes derived from amniotic fluid kidney progenitor cells from people with Alport syndrome, a hereditary kidney disease caused by a mutation in the

COL4 gene's alpha chain. This gene codes for a type IV collagen component. These chips had decreased albumin permeability, a symptom of Alport Syndrome, indicating that this system could be used to model kidney diseases.

23.4.4.2 Tubule-on-a-Chip

Many MPS models of proximal tubule epithelial cells (PTECs) have been developed. The proximal tubules are the primary site for transport-mediated reabsorption and secretion of xenobiotics. Researchers have developed a human proximal tubule-on-a-chip that replicates polarity, biomarker expression, biochemical and synthetic activity, and associated secretary and absorptive processes in the last few years [96, 97]. This model was evaluated for long-term viability, gene and protein expression, and vitamin D metabolism, but not renal clearance. Another study tested this model for pharmacokinetics in 2020, using five representative compounds as negative controls (as creatinine should not undergo resorption) and positive controls (as perfluorooctanoic acid is resorbed via active transport) [98]. To study renal uptake/resorption, researchers used a perfusion compound containing media. The chip was able to replicate tubular reabsorption and clearance in vivo. Due to the lack of a vascular channel, this device is best suited to determine the kinetics of non-secreted compounds like cadmium that actively absorb and accumulate in the kidney.

23.4.5 Brain-on-a-Chip

The brain, which is made up of the central nervous system and the spinal cord, is one of the most complex organs in the human body. The synapse, which allows a cell's axon to pass an electrical or chemical signal to a dendrite of another neuron, allows neurons to communicate with one or more neurons. Non-neuronal cell types, such as astrocytes, microglia, and oligodendrocytes, may also communicate with neurons. Neurodegenerative disorders can be caused by disruptions in these processes or changes in the neuronal network. For example, Alzheimer's disease is associated with neuronal cell death along with inflammation and accumulation of neurotoxic protein plaques [99]. In 2016, neurological disorders were estimated to be the leading cause of disability-adjusted life years and the second leading cause for mortality [100]. The failure rate of neurodegenerative disorder drugs is particularly high, where 99.6% of experimental drugs for Alzheimer's disease fail to reach the market [5, 101], highlighting the need for better human biology models. Around 2005, studies reported the fabrication of microfluidic chip containing microchannels with different heights and widths which could enable a compartmentalised culture where soma and axon could be separated. This allowed the researchers to study or treat specifically the axon or axonal regeneration post axotomy [102].

Myelin is a multilayered membrane produced by glial cells, including Schwann cells in the peripheral nervous system and oligodendrocytes in the central nervous system. Myelination is the process by which glial cells stretch and form myelin sheaths along the axons. This structure aids in a variety of neuronal activities,

including action potential propagation, improving the speed and efficiency of electrical communication. Many neuronal diseases are linked to demyelination or myelin loss, which can be caused by a variety of factors such as genetic mutations, chemical or environmental factors, inflammation, and so on. Recently, a study developed a three-dimensional peripheral nervous system (PNS) microfluidic platform that consists of primary Schwann cells and motor neurons that are co-cultured in a 3D hydrogen [103]. This system could mimic myelination, acute demyelination due to biochemical stimulation, and re-induce remyelination upon pharmacological intervention. The system could also be maintained for 40 days and was validated using known myelinating and demyelinating drugs.

The blood-brain barrier (BBB) is a selective barrier composed of brain microvascular endothelial cells (BMVECs) that controls the transport between blood and central nervous system (CNS). The BMVECs line the capillaries, surrounding extracellular matrix, pericytes, and astrocytes, leading to creation of a specific microenvironment that contributes to BBB function. A recent study developed an enhanced BBB-on-a-chip using human iPS-BMVECs that interface with primary human pericytes and astrocytes [104]. This chip displayed physiologically relevant human BBB functions for a week, such as low barrier permeability, expression of tight junction proteins, efflux pump, and transporters. Additionally, this chip also replicated a selective transcytosis of antibodies and peptides that have been previously observed in vivo.

23.5 Challenges and Considerations

While there have been significant advancements in the development of both single and multi-organ chips, there are certain challenges towards the large scale or high throughput use of organ-on-chips. In this section, we discuss the considerations that currently limit the widespread adoption of these methodologies in the preclinical stages of drug development.

23.5.1 Defining Context-of-Use

Researchers usually reverse-engineer the system when creating organs-on-chips, identifying the key components of a biological system required for a specific function and reassembling them. Instead of simulating the function of an entire organ, the system frequently represents a cross-section of one of the organ's major functional units. The lung alveolus chip, for example, which is made up of alveolar epithelium, endothelium, and immune cells, could be a good model system for studying pulmonary oedema and mechanisms to prevent pulmonary vascular leakage [105]. The addition of connective tissue cells, however, would be required to create a model system for pulmonary fibrosis. Thus, rather than replacing all animal models with organs-on-chip, specific organs-on-chip would be used to replace specific type of animal model or assay at a time. Thus, depending on the specific

research question being probed, single or multiple organs-on-chip would need to be modified.

In addition, the functional properties of an organ are often dictated not just by signalling pathways within the organ but also via endocrine or hormonal axis that links the functioning of multiple organs. This aspect is discussed in the following section.

23.5.2 Understanding Diurnal or Endocrine Fluctuations

Combining multiple organs-on-chips would also necessitate coupling them in such a way that they can mimic various diurnal or endocrine fluctuations and hormonal axis observed in vivo—which may not be a trivial task. Allometric scaling, control of flow rate, and cellular crosstalk would all be involved in the linking. Since hormonal fluctuations can affect cell and drug metabolism, cells and tissues responding to these cues also need to be present and wired properly. Organ-on-a-chip devices have been used to create some of these hormonal axes, such as the female menstrual cycle. In 2017, a study used murine ovarian follicles to develop a multi-unit microfluidic platform capable of displaying a 28-day menstrual cycle hormonal profile [106]. This system was able to simulate the endocrine loops between various organ modules such as the ovaries, fallopian tubes, uterus, cervix, and liver, as well as fluid flow between these tissues. This system, dubbed 'EVATAR', has a lot of promise in drug discovery research. However, modelling fluctuations in different hormones, which can often happen at the same time, is still challenging.

23.5.3 Cell Sourcing

The availability of renewable sources of cells for various systems is one of the major issues confronting organ-on-chip developers. Primary cell quality and quantity can vary depending on the supplier and, in some cases, even within the same batch. Furthermore, they can show significant donor-to-donor variation. Inter-donor variability, on the other hand, may be more clinically relevant because it resembles the variations seen in human populations. Because they can provide an infinite source of cells, iPSC lines are frequently viewed as a solution to a lack of primary cells. Additionally, multiple tissues for multi-organ-on-chip devices could come from the same donor. However, iPSC-derived cells' differentiation and maturation protocols can vary, resulting in a lack of reproducibility. As a result, these issues must be addressed by developing established and common protocols for validating each type of cell source for consistency, growth, and differentiation potential, as well as ensuring the cell supply chain.

23.5.4 Platform Fabrication

The fabrication material used to make the chip is also a critical parameter because it can affect how various compounds may bind or react with the cells and fluids in the system. Due to its affordability, ease of workability, and optical clarity, polydimethylsiloxane (PDMS) has been the most widely used polymer; however, it is gas permeable. This indicates that it could absorb small hydrophobic molecules as well. As a result, PDMS may not be the best material for drug discovery studies because it can absorb the drug or cause cross-contamination between chambers. Alternative materials, such as glass, silicon, and thermoplastics like cyclicolefin copolymer, are also being developed by researchers. However, with material selection, there is frequently a trade-off between availability, affordability, platform requirements, and ease of fabrication.

23.5.5 Scaling Up the Manufacturing Process

The issue of scaling up the manufacturing system must be addressed before these systems can be integrated into the drug development pipeline by pharma companies. Most organ-on-chip systems are currently developed in-house by academic institutions, and this process is constrained by cost, manufacturing equipment, and qualified personnel. As a result, standard operating procedures for designing and fabricating chips, as well as guidelines for good lab practices and quality control processes, must be documented so that manufacturers can easily replicate them. Furthermore, academic labs must scale up by forging partnerships with larger manufacturing firms. In addition, independent "qualification" labs, such as the National Center for Advancing Translational Sciences (NCATS) TissueChip Testing Centers in the USA or the European Union Reference Laboratory for Alternatives to Animal Testing in Europe, could be established to test the organ-on-chips being developed in academic labs.

23.5.6 Validating Organs-on-Chip

Validation is a crucial step on the path to regulatory acceptance of these technologies. Validation could be divided into three layers for various stakeholders. The first layer would entail evaluating the OoC system for sensitivity, precision, and specificity, which would indicate the system's level of repeatability. The second layer would involve assays to qualify the in vivo relevance. Whether animal model or human responses should be used as the gold standard during the process of validating data from OoC devices has been a point of contention. While animal models are currently the gold standard for drug development validation, the argument is that these devices would accurately reproduce human tissue responses, which may differ from those obtained using animal models. As a result, validation of these models by comparing data to data generated by animal models has been

argued against. Another possibility is to validate OoC data by comparing it to human clinical data; however, these ideas need to be explored further. The third layer would involve validation of the organ-on-chip data generated for proprietary compounds and submission of the data to relevant agencies. Microphysiological Systems Database, for example, collaborates with Tissue Chip Testing Centers, Technology developers, and commercial OoC suppliers to integrate the data into a public repository, which is funded by NIHNCATS.

23.6 Conclusions

A recent review was published by Donald Ingber with the title, "Is it time for reviewer to request human chip experiments instead of animal models?" [107]. With this provocative title, he argued that with the recent advances in the microphysiological systems, is the ask of reviewers for animal data—with their low physiological relevance to humans—still valid? While there cannot be a generic organ-on-chip device which would replace animal models, as these systems are defined on their context-of-use, they are also beginning to provide physiologically relevant answers to specific assays or questions regarding human diseases, which cannot be mimicked in animals. For example, animals are a poor model to understand many human neurological, stress-related, or lifestyle diseases. Due to their defined composition and specificity to humans or even a single patient, OoC devices can help in identifying key cellular and molecular components to biological pathways that are relevant to human physiology and pathophysiology. Nonetheless, to realise the full potential of these systems, many of the challenges mentioned in the chapter must still be addressed. With the co-ordinated efforts of different stakeholders, including technology developers, government validation bodies, pharma companies, manufacturing companies, replacement of animal models with Ooc systems during the preclinical testing, will be a foreseeable goal in the coming decade.

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References

1. Seok J et al (2013) Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 110:3507–3512
2. Sauter C, Wolfensberger C (1980) Interferon in human serum after injection of endotoxin. Lancet 2:852–853
3. Martignoni M, Groothuis GMM, de Kanter R (2006) Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol 2:875–894

4. Doncheva NT et al (2021) Human pathways in animal models: possibilities and limitations. Nucleic Acids Res 49:1859–1871
5. Mohs RC, Greig NH (2017) Drug discovery and development: role of basic biological research. Alzheimer's Dementia (NY) 3:651–657
6. Sin A et al (2004) The design and fabrication of three-chamber microscale cell culture analog devices with integrated dissolved oxygen sensors. Biotechnol Prog 20:338–345
7. Song JW et al (2005) Computer-controlled microcirculatory support system for endothelial cell culture and shearing. Anal Chem 77:3993–3999
8. Shin M et al (2004) Endothelialized networks with a vascular geometry in microfabricated poly(dimethyl siloxane). Biomed Microdevices 6:269–278
9. Lam MT, Huang Y-C, Birla RK, Takayama S (2009) Microfeature guided skeletal muscle tissue engineering for highly organized 3-dimensional free-standing constructs. Biomaterials 30:1150–1155
10. Jang K, Sato K, Igawa K, Chung U, Kitamori T (2008) Development of an osteoblast-based 3D continuous-perfusion microfluidic system for drug screening. Anal Bioanal Chem 390: 825–832
11. Huh D et al (2007) Acoustically detectable cellular-level lung injury induced by fluid mechanical stresses in microfluidic airway systems. Proc Natl Acad Sci U S A 104:18886–18891
12. Carraro A et al (2008) In vitro analysis of a hepatic device with intrinsic microvascular-based channels. Biomed Microdevices 10:795–805
13. Powers MJ et al (2002) A microfabricated array bioreactor for perfused 3D liver culture. Biotechnol Bioeng 78:257–269
14. Huh D et al (2010) Reconstituting organ-level lung functions on a Chip. Science 328:1662–1668
15. Yum K, Hong SG, Lee LP (2014) Physiologically relevant organs on chips. Biotechnol J 9:16–27
16. Pittman RN (2011) Regulation of tissue oxygenation. Morgan & Claypool Life Sciences, San Rafael
17. Shoemaker JT et al (2020) A 3D cell culture organ-on-a-Chip platform with a breathable hemoglobin analogue augments and extends primary human hepatocyte functions in vitro. Front Mol Biosci 7:296
18. Grant J et al (2021) Simulating drug concentrations in PDMS microfluidic organ chips. Lab Chip. https://doi.org/10.1039/D1LC00348H
19. Ucciferri N, Sbrana T, Ahluwalia A (2014) Allometric scaling and cell ratios in multi-organ in vitro models of human metabolism. Front Bioeng Biotechnol 2:74
20. E, G. et al (2014) Inflammation and cardiovascular disease: from pathogenesis to therapeutic target. Curr Atheroscler Rep 16:435
21. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420:860–867
22. Lee K-Y, Ito K, Maneechotesuwan K (2016) Inflammation to pulmonary diseases. Mediators Inflamm 2016:7401245
23. Shi K et al (2019) Global brain inflammation in stroke. Lancet Neurol 18:1058–1066
24. Hur J-Y et al (2020) The innate immunity protein IFITM3 modulates γ-secretase in Alzheimer's disease. Nature 586:735–740
25. Tsalamandris S et al (2019) The role of inflammation in diabetes: current concepts and future perspectives. Eur Cardiol 14:50–59
26. Beaurivage C et al (2020) Development of a human primary gut-on-a-chip to model inflammatory processes. Sci Rep 10:21475
27. VanDussen KL et al (2015) Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays. Gut 64:911–920
28. Trapecar M et al (2020) Gut-liver Physiomimetics reveal paradoxical modulation of IBD-related inflammation by short-chain fatty acids. Cell Syst 10:223–239.e9

29. Vos T et al (2020) Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the global burden of disease study 2019. Lancet 396: 1204–1222
30. Libby P et al (2019) Atherosclerosis. Nat Rev Dis Primers 5:56
31. Kehat I, Molkentin JD (2010) Molecular pathways underlying cardiac remodeling during pathophysiological stimulation. Circulation 122:2727–2735
32. Zhang B et al (2016) Biodegradable scaffold with built-in vasculature for organ-on-a-chip engineering and direct surgical anastomosis. Nat Mater 15:669–678
33. Huynh J et al (2011) Age-related intimal stiffening enhances endothelial permeability and leukocyte transmigration. Sci Transl Med 3:112ra122
34. Carrion B et al (2010) Recreating the perivascular niche ex vivo using a microfluidic approach. Biotechnol Bioeng 107:1020–1028
35. Whisler JA, Chen MB, Kamm RD (2014) Control of perfusable microvascular network morphology using a multiculture microfluidic system. Tissue Eng C Methods 20:543–552
36. Qiu Y et al (2018) Microvasculature-on-a-chip for the long-term study of endothelial barrier dysfunction and microvascular obstruction in disease. Nat Biomed Eng 2:453–463
37. Westein E et al (2013) Atherosclerotic geometries exacerbate pathological thrombus formation poststenosis in a von Willebrand factor-dependent manner. Proc Natl Acad Sci U S A 110: 1357–1362
38. Tovar-Lopez FJ et al (2010) A microfluidics device to monitor platelet aggregation dynamics in response to strain rate micro-gradients in flowing blood. Lab Chip 10:291–302
39. Costa PF et al (2017) Mimicking arterial thrombosis in a 3D-printed microfluidic in vitro vascular model based on computed tomography angiography data. Lab Chip 17:2785–2792
40. Blann AD, Lip GY (2001) Virchow's triad revisited: the importance of soluble coagulation factors, the endothelium, and platelets. Thromb Res 101:321–327
41. Lowe GDO (2003) Virchow's triad revisited: abnormal flow. Pathophysiol Haemost Thromb 33:455–457
42. van Hinsbergh VWM (2012) Endothelium - role in regulation of coagulation and inflammation. Semin Immunopathol 34:93–106
43. In vitro microvessels for the study of angiogenesis and thrombosis. PNAS. https://www.pnas.org/content/109/24/9342
44. Barrile R et al (2018) Organ-on-Chip recapitulates thrombosis induced by an anti-CD154 monoclonal antibody: translational potential of advanced microengineered systems. Clin Pharmacol Ther 104:1240–1248
45. Improving and accelerating drug development for nervous system disorders. Neuron. https://www.cell.com/neuron/fulltext/S0896-6273(14)00905-2?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0896627314009052%3Fshowall%3Dtrue
46. Choi DW et al (2014) Medicines for the mind: policy-based 'pull' incentives for creating breakthrough CNS drugs. Neuron 84:554–563
47. Takeda S et al (2015) Neuronal uptake and propagation of a rare phosphorylated high-molecular-weight tau derived from Alzheimer's disease brain. Nat Commun 6:8490
48. Nobuhara CK et al (2017) Tau antibody targeting pathological species blocks neuronal uptake and interneuron propagation of tau in vitro. Am J Pathol 187:1399–1412
49. Choi YJ et al (2013) Neurotoxic amyloid beta oligomeric assemblies recreated in microfluidic platform with interstitial level of slow flow. Sci Rep 3:1921
50. Park J et al (2018) A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. Nat Neurosci 21:941–951
51. Prots I et al (2018) α-Synuclein oligomers induce early axonal dysfunction in human iPSC-based models of synucleinopathies. Proc Natl Acad Sci U S A 115:7813–7818
52. Fernandes JTS, Chutna O, Chu V, Conde JP, Outeiro TF (2016) A novel microfluidic cell co-culture platform for the study of the molecular mechanisms of Parkinson's disease and other Synucleinopathies. Front Neurosci 10:511

53. Bolognin S et al (2019) 3D cultures of Parkinson's disease-specific dopaminergic neurons for high content phenotyping and drug testing. Adv Sci 6:1800927
54. Kerman BE et al (2015) In vitro myelin formation using embryonic stem cells. Development 142:2213–2225
55. Licht-Mayer S et al (2020) Enhanced axonal response of mitochondria to demyelination offers neuroprotection: implications for multiple sclerosis. Acta Neuropathol 140:143–167
56. Hyung S et al (2019) Optogenetic neuronal stimulation promotes axon outgrowth and myelination of motor neurons in a three-dimensional motor neuron-Schwann cell coculture model on a microfluidic biochip. Biotechnol Bioeng 116:2425–2438
57. Gould SE, Junttila MR, de Sauvage FJ (2015) Translational value of mouse models in oncology drug development. Nat Med 21:431–439
58. Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J (2014) Clinical development success rates for investigational drugs. Nat Biotechnol 32:40–51
59. Montanez-Sauri SI, Sung KE, Berthier E, Beebe DJ (2013) Enabling screening in 3D microenvironments: probing matrix and stromal effects on the morphology and proliferation of T47D breast carcinoma cells. Integr Biol (Camb) 5:631–640
60. Regier MC et al (2016) Transitions from mono- to co- to tri-culture uniquely affect gene expression in breast cancer, stromal, and immune compartments. Biomed Microdevices 18:70
61. Hassell BA et al (2017) Human organ Chip models recapitulate Orthotopic lung cancer growth, therapeutic responses, and tumor dormancy in vitro. Cell Rep 21:508–516
62. Folkman J, Watson K, Ingber D, Hanahan D (1989) Induction of angiogenesis during the transition from hyperplasia to neoplasia. Nature 339:58–61
63. Nguyen D-HT et al (2013) Biomimetic model to reconstitute angiogenic sprouting morphogenesis in vitro. Proc Natl Acad Sci U S A 110:6712–6717
64. Phan DTT et al (2017) A vascularized and perfused organ-on-a-chip platform for large-scale drug screening applications. Lab Chip 17:511–520
65. Jeong S-Y, Lee J-H, Shin Y, Chung S, Kuh H-J (2016) Co-culture of tumor spheroids and fibroblasts in a collagen matrix-incorporated microfluidic Chip mimics reciprocal activation in solid tumor microenvironment. PLoS One 11:e0159013
66. Rizvi I et al (2013) Flow induces epithelial-mesenchymal transition, cellular heterogeneity and biomarker modulation in 3D ovarian cancer nodules. Proc Natl Acad Sci U S A 110:E1974–E1983
67. Reymond N, d'Água BB, Ridley AJ (2013) Crossing the endothelial barrier during metastasis. Nat Rev Cancer 13:858–870
68. Zervantonakis IK et al (2012) Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. Proc Natl Acad Sci U S A 109:13515–13520
69. Tang Y et al (2017) A biomimetic microfluidic tumor microenvironment platform mimicking the EPR effect for rapid screening of drug delivery systems. Sci Rep 7:9359
70. Akabane T et al (2010) A comparison of pharmacokinetics between humans and monkeys. Drug Metab Dispos 38:308–316
71. Sharma V, McNeill JH (2009) To scale or not to scale: the principles of dose extrapolation. Br J Pharmacol 157:907–921
72. Cao X et al (2006) Why is it challenging to predict intestinal drug absorption and oral bioavailability in human using rat model. Pharm Res 23:1675–1686
73. McAleer CW et al (2019) On the potential of in vitro organ-chip models to define temporal pharmacokinetic-pharmacodynamic relationships. Sci Rep 9:9619
74. Herland A et al (2020) Quantitative prediction of human pharmacokinetic responses to drugs via fluidically coupled vascularized organ chips. Nat Biomed Eng 4:421–436
75. Novak R et al (2020) A robotic platform for fluidically-linked human body-on-chips experimentation. Nat Biomed Eng 4:407–420
76. Onakpoya IJ, Heneghan CJ, Aronson JK (2016) Worldwide withdrawal of medicinal products because of adverse drug reactions: a systematic review and analysis. Crit Rev Toxicol 46:477–489

77. Kaese S, Verheule S (2012) Cardiac electrophysiology in mice: a matter of size. Front Physiol 3:345
78. Finding the rhythm of sudden cardiac death. Circ Res. https://www.ahajournals.org/doi/full/10.1161/circresaha.116.304494
79. Li J, Patel VV, Radice GL (2006) Dysregulation of cell adhesion proteins and cardiac arrhythmogenesis. Clin Med Res 4:42–52
80. Tanaka Y et al (2007) A micro-spherical heart pump powered by cultured cardiomyocytes. Lab Chip 7:207–212
81. Gupta AC et al (2018) Establishment of an in vitro organoid model of dermal papilla of human hair follicle. J Cell Physiol 233:9015–9030
82. Zhang X, Wang T, Wang P, Hu N (2016) High-throughput assessment of drug cardiac safety using a high-speed impedance detection technology-based heart-on-a-Chip. Micromachines (Basel) 7:122
83. Olson H et al (2000) Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 32:56–67
84. Monticello TM et al (2017) Current nonclinical testing paradigm enables safe entry to first-in-human clinical trials: the IQ consortium nonclinical to clinical translational database. Toxicol Appl Pharmacol 334:100–109
85. Delalat B et al (2018) Microengineered bioartificial liver Chip for drug toxicity screening. Adv Funct Mater 28:1801825
86. Greuter T, Shah VH (2016) Hepatic sinusoids in liver injury, inflammation, and fibrosis: new pathophysiological insights. J Gastroenterol 51:511–519
87. A 3D microfluidic liver model for high throughput compound toxicity screening in the OrganoPlate®. Elsevier Enhanced Reader. https://reader.elsevier.com/reader/sd/pii/S0300483X20303061?token=292AC81EE0D39F4E323718BC0ADA6ACD317C9DB2A8D6BC17BA005384713E0AAB4577E4D8699B40C0FB334EFD4A80EA58&originRegion=eu-west-1&originCreation=20210912142049. https://doi.org/10.1016/j.tox.2020.152667
88. Jang K-J et al (2019) Reproducing human and cross-species drug toxicities using a liver-Chip. Sci Transl Med 11:eaax5516
89. Stucki AO et al (2015) A lung-on-a-chip array with an integrated bio-inspired respiration mechanism. Lab Chip 15:1302–1310
90. Zamprogno P et al (2021) Second-generation lung-on-a-chip with an array of stretchable alveoli made with a biological membrane. Commun Biol 4:168
91. Huang D et al (2021) Reversed-engineered human alveolar lung-on-a-chip model. Proc Natl Acad Sci U S A 118:e2016146118
92. Heinonen IHA, Sorop O, Merkus D, Duncker DJ (2017) Comparative physiology and pathophysiology of the coronary circulation. In: Escaned J, Davies J (eds) Physiological assessment of coronary stenoses and the microcirculation. Springer, New York, pp 287–294. https://doi.org/10.1007/978-1-4471-5245-3_21
93. Chu X, Bleasby K, Evers R (2013) Species differences in drug transporters and implications for translating preclinical findings to humans. Expert Opin Drug Metab Toxicol 9:237–252
94. Metcalfe W (2007) How does early chronic kidney disease progress? A background paper prepared for the UK Consensus Conference on early chronic kidney disease. Nephrol Dial Transplant 22 Suppl 9:ix26–30
95. A glomerulus-on-a-chip to recapitulate the human glomerular filtration barrier. Nat Commun. https://www.nature.com/articles/s41467-019-11577-z
96. Chang S-Y et al (2017) Human liver-kidney model elucidates the mechanisms of aristolochic acid nephrotoxicity. JCI Insight 2:95978
97. Weber EJ et al (2016) Development of a microphysiological model of human kidney proximal tubule function. Kidney Int 90:627–637
98. Sakolish C et al (2018) Technology transfer of the microphysiological systems: a case study of the human proximal tubule tissue chip. Sci Rep 8:14882
99. Masters CL et al (2015) Alzheimer's disease. Nat Rev Dis Primers 1:1–18

100. Feigin VL, Vos T (2019) Global burden of neurological disorders: from global burden of disease estimates to actions. Neuroepidemiology 52:1–2
101. Pistollato F et al (2016) Alzheimer disease research in the 21 st century: past and current failures, new perspectives and funding priorities. Oncotarget 7:38999–39016
102. Taylor AM et al (2005) A microfluidic culture platform for CNS axonal injury, regeneration and transport. Nat Methods 2:599–605
103. Hyung S et al (2021) A 3D disease and regeneration model of peripheral nervous system–on–a–chip. Sci Adv 7:eabd9749
104. Park T-E et al (2019) Hypoxia-enhanced blood-brain barrier Chip recapitulates human barrier function and shuttling of drugs and antibodies. Nat Commun 10:2621
105. Huh D (2015) A human breathing lung-on-a-Chip. Ann Am Thorac Soc 12:S42–S44
106. Xiao S et al (2017) A microfluidic culture model of the human reproductive tract and 28-day menstrual cycle. Nat Commun 8:14584
107. Ingber DE (2020) Is it time for reviewer 3 to request human organ Chip experiments instead of animal validation studies? Adv Sci 7:2002030

Application of Organ-on-Chip in Blood Brain Barrier Model

24

P. Vatsa and A. B. Pant

Abstract

The blood–brain barrier (BBB) is one of the discerning endothelial barriers of the human body because of its selective permeability for the molecules being directed to the brain. Therefore, acquiring a clear understanding of the physiological properties of the BBB becomes necessary to advance in the field of physiological modelling and therapeutics in fine fettle and brain pathologies. In this perspective, numerous in vivo and in vitro models have been established till now and these have, to a great extent, provided insights into the attributes and transport systems embraced by the human brain. However, they have failed to provide a firm ground for translating the insights into therapeutic outcomes. Contemporary advances towards the progress of pragmatic in vitro BBB models, mimicking its physiology, include the development of brain organoids, spheroids, organs-on-a-chips (OOCs), 3D printed microfluidics, or a combination of any of these. OOCs have typically enticed a substantial interest in the current decade towards modelling of the BBB as it might even prove to replace the animal model systems in the long run. The advances in this direction have opened up new avenues, especially in the fields of disease modelling, neurotoxicity screening, drug discovery, and personalized medicine. In this chapter, we aim to portray a glimpse of the organ-on-a-chip in the area of blood-brain barrier modelling, with its existing manoeuvres and caveats in their implementation, and attempt to highlight its importance for the coming decade.

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24.1 Introduction

24.1.1 Organ-on-a-Chip

OOAC is really a biomimetic system capable of simulating the environment of a physiological organ and regulating important parameters, such as concentration gradients, shear force, cell patterning, tissue borders, and tissue–organ interactions. OOAC's main purpose is to mimic the physiological milieu of human organs [1]. Microfluidics is a modern science for manipulating and processing microscale fluids with extreme precision. A "lab-on-a-chip" is a device that is often used to accurately manage microfluidic (10^{-9} to 10^{-18} L) fluids, utilizing channels ranging in size from tens to hundreds of microns [2]. The microchannel is narrow, but it has a large surface area and high mass transfer, making it ideal for microfluidic technology applications that require low cost, controlled volumes, quick mixing rates, rapid responses, and precision control of physical and chemical characteristics. Sample preparation, reactions, separation, detection, and fundamental operational units such as cell culture, sorting, and cell lysis are all integrated into microfluidics. Because of these factors, interest in OOAC has grown. OOAC incorporates a variety of chemical, biological, and material scientific fields and was named one of the World Economic Forum's "Top Ten Emerging Technologies" [1]. The applications of an OOC across various research areas are briefly alluded in Fig. 24.1.

24.1.2 Background: History, Structure, and Functions of the Blood Brain Barrier (BBB)

Lina Stern and Raymond Gautier named the blood brain barrier (BBB) as "la barrière hémato-encéphalique" in 1921 [3]. Later, Ehrlich used a formulation consisting of trypan blue and potassium ferrocyanide and found that the brain tissue is separated from the circulatory system via a barrier that is impermeable to nutrients or drugs [4]. Much later, Hugo Spatz came across the presence of non-perforated endothelial cells which cast the properties of the blood brain barrier [3]. In 1967, Reese and Karnovsky observed the accumulation of horseradish peroxidase in the lumen of brain capillaries using electron microscopy and settled the current paradigm revolving around the BBB and established that endothelial tight junctions (TJs) confer the BBB its unique barrier-like attributes [5]. The human body in itself is an overly complex and organized structure further composed of even more sophisticated sub-systems having multifaceted dynamic networking. BBB is one such intricate network of microvessels lined by vascular endothelial cells (ECs) in association with pericytes, extracellular matrix (ECM), neurons, and glial cells. These specialized ECs have unique barrier attributes which make the BBB microvasculature quite distinguishable from the rest of the body [6]. The capillaries here have weaker pinocytosis [7] due to the presence of tight junctions and run in a tightly welded space.

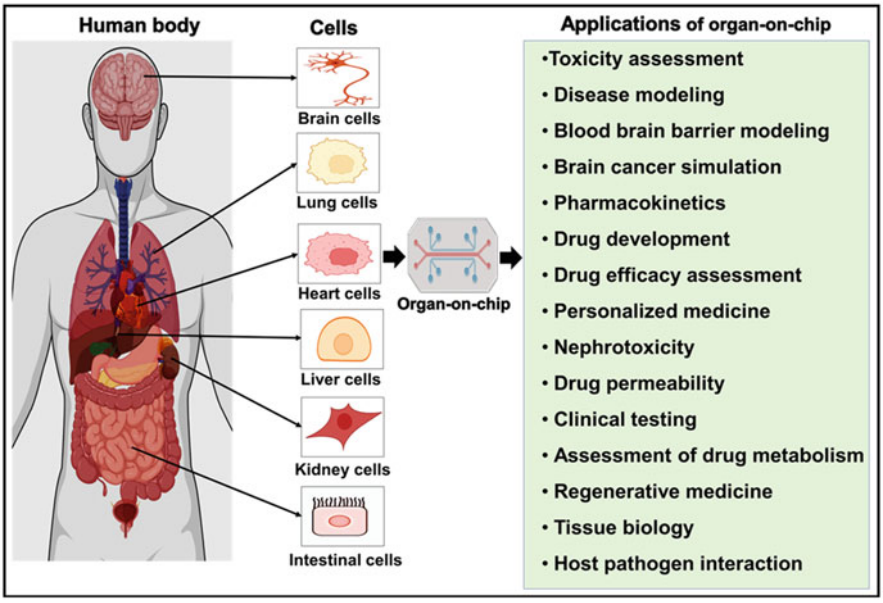

Fig. 24.1 Applications of organ-on-chip systems depicted schematically: These systems provide a wide range of applications, including disease modelling, organogenesis investigations, drug discovery, and personalized medicine, by combining tissue engineering, microfabrication, and stem cell biology approaches into organ-on-chip technology. (Figure created using Biorender.com)

The entry of peripheral substances into the central nervous system (CNS) is highly limited because of the presence of these tight junctions between the cerebral capillary ECs, its continuous outer basement membrane, and the terminal foot of glial cells on its outer vessel walls [8, 9]. Due to the development and presence of BBB over the due course of evolution, numerous pathogenic entries are restricted to the CNS that aid in the normal physiological functioning of the CNS. The BBB works as a dynamic and functional interface between the systemic circulation and the CNS [10]. Its role is to maintain homeostasis in the brain environment and provide protection against the entry of potentially harmful and unwanted bacteria, viruses, exogenous substances, endogenous cellular cargoes, and also against fluctuations of the system [11, 12]. It is also responsible for regulating the transport of nutrients and molecules required for the optimum functioning of the CNS [13]. It also plays a vital role in responding to various pathological and physiological cues like oxidative stress, inflammatory molecules/signals, rheological changes, acute brain injury, etc. [14–16]. The complex interplay between BBB endothelium and the cellular milieu around it, comprising pericytes, neurons, microglia, astrocytes and endothelial glycocalyx together, are termed as neurovascular unit (NVU) [17–19]. The extracellular matrix proteins secreted by ECs and astrocytes make up the basement membrane and it is important for cellular support and signal transduction [20–23]. The endothelial cells of BBB have limited pinocytosis, absence of perforations,

asymmetry between lumen and albumen of trans-membrane transport and efflux systems that regulate the trafficking of substances between the brain parenchyma and blood [7]. TJ proteins such as occludins, claudins constrain the paracellular efflux of ions and hydrophilic solutes through the BBB [24] causing high electrical resistance with readings $\geq$1800 Ω cm^2 as measured in situ in rats [25]. TJs also function as a barricade that restricts the free passage of lipids and proteins in the cellular membrane between the apical and the basal surfaces. This specialized carrier-mediated system aids in the delivery of water soluble, biologically vital molecules such as amino acids, glucose, etc. into the brain [7].

Adherent junctions (AJ) and gap junctions (GJ) also play an important role in imparting tightness to the BBB and help in intercellular adhesion and communication [26]. Efflux transporters, like P-glycoprotein (Pgp) [27], breast cancer resistance protein (BCRP) [28], and multidrug resistance-related proteins (MRPS) [29], as well as cytochrome P450 (e.g. CYP3A4, NADPH-CYPP450 reductase, etc.) [30] and Phase II detoxifying enzymes (such as UGT1A4), which help protect the brain from potentially harmful substances [27], are also present on the BBB endothelium. This defence mechanism that imparts protection to the brain from numerous harmful chemicals plays hindrance for drug delivery into the brain. Certain studies have shown that around 98% of small molecules and 100% large molecules cannot pass through the BBB [31, 32]. Thus, the BBB is also a crucial hurdle encumbering the treatment of key neurological ailments [33]. A breakdown of the BBB, linked to heightened BBB permeability and de-regulated influx and efflux, results in toxins and immune cells infiltrating the CNS [20]. Several diseases are associated with the dysfunctioning of the BBB system, such as multiple sclerosis, stroke, brain tumour, bacterial meningitis, and Alzheimer's disease [12, 34], which influence up to one billion people worldwide and correspond to 10.9% of the global problem in high income countries [35]. Thus, the development of physiologically relevant platforms that can accurately mimic BBB phenotypes, and allow observing of complex intercellular communications in the BBB, could advance our understanding of neurological diseases as well as the progress in the field of designing new therapies and help in identifying xenobiotics that may impart toxic effects on the brain.

24.2 Models for Blood Brain Barrier

The three most conventional methods to analyse the transport across the BBB are animal-based model systems, parallel artificial membrane permeability assays (PAMPA) [36], and transwell assays [37]. Animal-based model systems have their own drawbacks, as their outcomes are not 100% applicable in human relevance [38], and the other two are too simplified to physiologically replicate the BBB physiology. The advancements in the field of microfluidics and fabrication technologies have led to the development of in vitro organ-on-chips-(OOC) based model systems; like the first of its kind examples of organ-on-the-chip, like a lung-on-a-chip, atherosclerosis-on-a-chip, etc., provide the evidence that OOC can replicate the organ system in a human body and can be used to study physiological responses that could not be

studied before [39, 40]. The use of microfluidics in BBB-based models can aid the research advances by providing much more accurate measurements and geometries and by exposing the endothelium lining to the physiological fluids having molecules of our interest [41].

24.3 Microfluidics-Based Designing

24.3.1 Principles of Microfluidic Device Design

Any standard in vitro BBB model should have features that are similar to in vivo conditions, viz. (1) cellular interactions, (2) vascular structure, (3) permeable base membrane, (4) ECs having shear stress. The microfluidic systems comprising these attributes have overcome the limitations of 2D models such as cellular morphology and gene expression [42]. Cellular interactions have a key role in tissue repair and regeneration, thereby making it a crucial factor while designing any OOC [10]. The major challenge faced by researchers in designing BBB chips is replicating the permeable basal membrane, which is essential for major cellular processes like homeostasis, differentiation, cellular integrity, etc. The ideal thickness of the artificial basal membrane should be ~100 nm and should be composed of biocompatible materials [21, 43]. In this aspect, various BBB models, having different strategies, designs, and materials [44], have been generated so far, as discussed below.

24.3.1.1 Sandwich Design

The initial traditional transwell designs [45] for organ-on-chip evolved into sandwich design comprising two channels—upper and lower—composed of polydimethylsiloxane (PDMS) [46–50]. The upper and lower channels are separated from each other by a porous membrane, thus giving it a sandwich-like structure. Typically, polycarbonate membranes have a pore size diameter range of 0.2–3 μm. The ECs are seeded in the upper layer and other cells like pericytes, astrocytes, etc. are seeded into the lower channel. These model systems have some major drawbacks, like difficulty with high-resolution imaging and real-time monitoring of cell proliferation and communication because of factors like lack of transparency of membrane and cell seeding. The use of transparent membranes such as those made up of polytetrafluoroethylene has helped a bit towards improving such models [50]. The height of the channel is another major concern for this model as the cell-to-cell contact between the upper and lower channels is inhibited due to higher separation. For this, an alternative approach has been used that has helped in improving cell-to-cell contact. Here, the ECs are cultured in the lower channel in a 3D vessel-like structure and other cells are seeded in the upper channel [51, 52].

24.3.1.2 Parallel Design

In this model, two horizontally separated channels are aligned in a planar parallel configuration, separated by microchannels composed of PDMS. It differs from the former as it has a PDMS-based microchannel, such as the one designed by Prabhakar

Pandian et al. They separated the brain and the blood compartment with an array of micropillars gapped at 3 μm and then utilized this model for co-culturing with astrocytes. This model has provided improved cellular interactions and imaging outcomes, but lacks in reducing the thickness of the PDMS membrane to that of the native basal membrane [53, 54]. To mimic the basal membrane, ECM gel-based barriers like hydrogels pre-mixed with astrocytes or neurons have been used. The outcomes have shown that these hydrogels can be used as ECM support and physical barrier [55]. The hydrogel-based gel barriers have demonstrated an enhanced resemblance to the basal membrane in regard to the composition, stiffness, and thickness. Xu et al. have used collagen gel-based materials in their study and have shown similar results [56].

24.3.1.3 3D Tubular Structure Design

PDMS-based models have been shown to have compromised flow and shear stress due to their fabrication, causing morphological and physiological differences in the ECs seeded into the channels. To overcome this, van der Helm et al. constructed cylindrical microchannels which made the inner walls resistant to shear stress [57]. Chrobak et al. designed a collagen, a-gel-based 3D microvascular tube-like structure that comprised microneedles of diameter 75–100 μm [58], and this was later used by Kim and colleagues in defining their BBB model [59]. These provided an advantage in applying different fluid flow rates as the diameter of these microtubes could be modulated. The 3D micro-vascular tubes were then co-cultured with astrocytes or pericytes entrenched in a collagen matrix [28]. Intriguingly, ECs were found to be secreting basal laminin, implying that the ECM gel integrated into the BBB can expedite the development of a natural BM [28, 60, 61]. However, ECM gel-based 3D tubular BBB devices possess restrictions over the difficulty to include barrier rigidity measurement because of the cylindrical chip design [28].

24.3.1.4 Vasculogenesis Design

Major BBB designs concentrate on manufacturing microvessels compliant to pre-determined scaffolds such as are present in microfluidic channels or microneedles [62]. Vasculogenesis strategy has been employed in the BBB systems to give rise to new microvessels. Like a collagen matrix was incorporated into the microfluidic device near the embedded ECs that led to formation of new micro vessels [31]. Bang et al. used this approach for creating a BBB where the device was composed of dual channels for ECs and neuronal cells divided via fibrin hydrogel. This eventually led to formation of networks within the fibrin hydrogel and a connection was established with the astrocytes and neurons present [32]. This in vivo-like BBB can recapitulate the interaction between the ECs and the pericytes and the astrocytes. However due to irregular branching of the newly formed vessels, the experimental reproducibility and application of this BBB model were compromised.

24.4 Models for the BBB

24.4.1 In Vivo Models

A great deal of our knowledge on the subject of BBB firstly came from the use of animal models in the field, which explicated the basic underlying mechanisms of BBB permeability, transport across BBB, its architecture and associated features [63–65]. The benefit from these models was their physiological relevance in terms of structure and blood flow, thereby these animal models provided enormous insights into brain tissue physiology. Nevertheless, the challenge of extrapolating these animal model-based findings to the translational human applications remained a formidable challenge in front of the researchers due to the underlying genetic, immunological, and molecular differences between the two species [66, 67]. Another major concern with these models were: exorbitant and ethical issues that incited the studies in the BBB field towards in vitro model systems.

24.4.2 In Vitro Models

24.4.2.1 Cell Sources for Development of In Vitro Models

The cell source selection is indispensable for the development of in vitro BBB model systems. The major facets include accessibility, relevance to human systems, disbursements, resilience, barrier properties, reproducibility, etc. [68]. For any BBB model to be successful, choosing the right species as a cell donor is crucial. For instance, donor species like the humans themselves, such as porcine, bovine, murine, or simian, can be used as a source of primary cells, differentiated cells, or induced pluripotent stem cells (iPSCs) [69]. BBB in vitro model systems also need a robust selection of endothelial cells (along with astrocytes, pericytes, neurons, etc.), as they differ from other ECs in terms of morphology and functions, like the presence of tighter cellular junctions, high polarization, high transepithelial/transendothelial electrical resistance (TEER), or low permeability [70–72]. To engineer BBB, other key points to consider are the transporter receptors (trs) like GLUT-1 or Pgp [73], and tight junction proteins, like occludins and claudins [74].

Primary ECs can be derived from biopsies (animal/human) and propagated in vitro, and these are highly accessible, used, and well documented in the literature [31]. Although cells derived from animal systems are not similar to the human cells *w.r.t.* their composition or function, and yet have to be used, as human primary cells from donors (a preferred choice) are not always available for use or have certain revolving ethical concerns. Human-derived cells express high levels of platelet endothelial cell adhesion molecule 1 (PECAM-1), claudin-5, and zonula occludens 1 (ZO-1), but have low TEER values (under 100 Ω/cm^2), and they often tend to lose their BBB properties in eventual passages. The heterogeneity of the cells derived from human source also lowers the authenticity and reproducibility of the results [75–77]. The endothelial cells can modify their phenotype on the availability of the co-culture environment and the stromal cells used in the respective model. For

example, human umbilical vein endothelial cells (huvECs), when co-cultured with neurons, astrocytes, or pericytes, have shown expression of ZO-1 and VE-cadherin, key expression markers of BBB and generate a stable vascular function and barrier function [78–81].

With the limitations with primary cultures, the use of immortalized cell lines gained popularity in the development of BBB models, as they had low cost and were comparatively easier to maintain under in vitro conditions. These are genetically identical to each other and retain their functionality over the passages, thus ameliorating the stability and reproducibility of the model [82]. However, these types of cells have low BBB properties and compromised barrier function and may not be suitable for studies revolving around molecular transport in the BBB [83, 84]. Cell lines like TY10 express elevated levels of claudin-5 and VE-cadherin and hcmec/D3 have higher ZO-1 and absence of claudin-5. This suggests that human cell lines have inconsistent protein levels and their use would vary from model to model depending upon the requirement which might affect the outcome of various studies and establishing links [85, 86]. The lack of BBB like properties, i.e. they are incapable of making a tight layer of cells, make these cells unfit for use to a certain extent, but can be used in studies like drug absorption or host-pathogen interactions in and across the BBB [3].

In the past decade, cells derived from the embryonic origin (Embryonic stem cells [ESCs]) or mesenchymal stem cells (MSCs) or iPSCs have gained popularity in brain-related pathologies and mechanistic studies [87–91]. ESCs like H1 and H9 for PECAM-1 expression [92], and HES3 and HES4 are utilized for VE-cadherin expression [93], can be differentiated into the brain ECs or embryoid body using various protocols for BBB modelling [94]. However, the yield from such methods is low and the area needs more detailed studies for expansion in the BBB research. The development of iPSCs by Yamanaka et al. [95] has greatly benefitted the development of in vitro model systems. The iPSCs can yield numerous cell lines under specific conditions whilst maintaining homogeneity and also possess exceptional barrier properties with the expression of ZO-1, occludin, and claudin-5. Their permeability and TEER values are quite comparable to that of in vivo model systems [68, 96]. The limitation lies in the validation and acceptance of a single protocol to produce ECs from iPSCs, the life span of iPSC culture, the length and variation of steps, and use of numerous external stimuli-like growth factors in the production of ECs for BBB studies [97–99].

24.4.3 In Vitro Model Systems

Since the 1980s, the major challenge for BBB modelling has been the development of such model systems that have transport properties moderately comparable to human BBB. With the evolution in the complexity of BBB models, numerous platforms have been developed like that with multiple interacting cell types or 3D hydrogel-based BBB models. These can be categorized as 2D static models and 3D models as discussed below.

24.4.3.1 2D Static Model

The cells, when cultured on a hard plastic surface in transwell in absence of flow, comprise the 2D static models. The first-ever model of BBB comprising ECs isolated from brain capillaries and monoculture, developed by Bowman et al., showed the structure of TJs via freeze-fracture [31]. Further, to mimic the BBB micro-environment and decrease the stimuli from neighbouring cells, co-culture models were developed using ECs, astrocytes, neurons, and pericytes [100]. This model had advantages like easy imaging, control over environmental conditions, but it had several limitations such as BBB metabolomics, diffusion or permeability studies were difficult to assess due to lack of interface between vascular and parenchymal components. The plastic tissue culture base was flat and lacked a layering system that limits the cell development and lacked in presence of extracellular matrix required, resulting in limited potential and clinical translation of the findings [101].

The Transwell system overcomes these challenges and allows to conduct of permeability assays, cell migration via the membrane, promoting epithelial cell polarity, or studying multicellular interactions [71]. This system allows for the integration of at least two types of cells, ECs in the top and others, like pericytes, neurons, in the other (direct contact or indirect contact with ECs) [102]. Primary cells, iPSCs, cell lines have been used in the BBB Transwell model system and this has provided access to assess the TEER and solute permeability of BBB [103]. The limitations lie in the flat cast, which constrains the cellular interactions, which might be responsible for promoting barrier properties and result in inefficient BBB modelling [104]. Currently, hybrid models constituting 3D gels on Transwell inserts have been created and these are called chip-on-a-Transwell systems [3].

24.4.3.2 2D Organ-on-a-Chip

The previously established in vitro models for BBB studies did not provide cell-to-cell interaction, lacked in accurate assessment of hemodynamics, or lacked in properly organized micro-environment [105]. These limitations have been overcome by the introduction of Organ-on-Chip (OOC) models which are compartmentalized and have membranous separations to mimic the BBB microenvironment. These models aid in providing real-time read-outs along with understanding cellular interactions, fluid flow, and shear stress within BBB [106]. Organ on a chip is a biomimetic system that mimics the physiology of an organ, along with the capability to control vital constraints, including concentration gradients, shear force, cell patterning, tissue-boundaries, and tissue-organ interactions. The foremost target of any OOC is to mimic the physiological environment of human organs. 2D blood-brain-barrier-on-a-chip models (BBOCs) can be defined as microfluidic chips that constitute of two or more BBB cells with hydrogels of 100 μm thickness. These provide a greater physiological resemblance to the BBB and can facilitate cell migration and angiogenesis [107, 108]. These serve as powerful gizmos that can utilize primary cells or iPSCs to study the BBB physiology such as expression analysis of TJs, TRS, and adherence junctions (AJS) that impart to the BBB its attributes [99, 108, 109]. Recent advancements in this model have enabled in production of a neurovascular unit platform using cells of the central nervous system

to study metabolic pathways between brain parenchyma and vasculature [51, 110]. Also, researchers have successfully designed complex models comprising multiple organs like the brain, intestine, liver, kidney, and skeletal muscles on an OOC to be used in pharmacological studies [111, 112]. These models face limitations when large scale studies are carried out and miniaturization of these systems [113, 114].

24.4.3.3 3D BBB Models

3D model systems can mimic the natural organ systems' architecture in a much more efficient manner by having a much greater physiological resemblance. Ahn et al. developed a 3D model with astrocytes at the base seeded in Matrigel and ECs and pericytes in a microfluidic device. They observed that the astrocytes extended their end feet towards the pericytes and the ECs similar to in vivo condition [115]. 3D monolayer models have been very beneficial because of their physiological relevance in generating high throughput in in vitro models for studies like metastasis or drug transport in the BBB [56]. The ECs cultured under 3D platforms have shown to have better TJ expression and enhanced TEER value, indicating that usage of adequate matrices in designing plays a key role in determining the integrity and function of BBB model [116]. Numerous 3D BBB are available, like 3D channels embedded in or adjacent to hydrogel compartments that provide desired attributes for morphology, high throughput capability, cellular organization, and superior barrier function. It consists of hollow tubular structure, which is lined with ECs and other cells embedded in the hydrogel [117]. Other such structures have been developed similar to these such as one composed of hydrogels having better physiological relevance and can incorporate certain other cells of the BBB like pericytes and astrocytes [118]. The use of multichannel microfluidic chips by Brown et al. and Adriani et al. comprised ECs derived from human brain along with pericytes, astrocytes, and neurons embedded in adjacent collagen hydrogel [49, 55]. As collagen is not naturally present in the brain parenchyma so it can be substituted with hyaluronic acid- or proteoglycan-based hydrogels for efficacious results [3]. These hydrogels have large diameters (~500 μm) and larger spaces between the stromal cells, which compromises its replication to the BBB. These limitations have been addressed by several groups recapitulating the BBB vascular system with small diameter lumens and closely assembled ECs [119].

The in vivo process of formation and assembly of the BBB comprises vasculogenesis and angiogenesis. The ECs can form hollow tubular structures and hence are employed in 3S vasculature systems to recapitulate the BBB. The ECs, along with Human umbilical vein endothelial cells (hUVECs) and pericytes from placenta or stromal cells of lungs, have resulted in a BBB model having a better morphology expression of TJ proteins and an improved transport mechanism for the solutes, when compared to the 2D models or 3D tubular models [120, 121]. A model by Bang and his colleagues used hUVECs fibroblasts and astrocytes and neurons to mimic the BBB in 3D. This model offered deep insights into the role of astrocytes in imparting the BBB its properties [122].

The development of 3D-OOC models face a major issue wherein to maintain the EC properties isolated from various tissues. The brain microvascular endothelial cells (BMECs) obtained from brain biopsies are known to retain their self-lumenizing properties if propagated in appropriate 3D hydrogels. This property of BMECs was used by Lee et al. in developing their model. They used BMECs, pericytes, and astrocytes from brain and lung fibroblasts to create a self-assembled 3D chip that had enhanced barrier permeability and increased TJ protein expression [107]. The current BBB models regress within a couple of weeks, making it difficult to plan long-term studies. Today the 3D brain organoids can be developed from iPSCs or ESCs or Pluripotent Stem Cells (PSCs). These bear the capacity to give rise to brain regions like cerebral cortex, etc. and hence recapitulate the BBB quite well [123]. For Alzheimer disease models, patient-derived iPSCs have also been used [124]. The generation of BBB does not seem to be a problem now; however, the lack of proper vasculature in these still persists. Pham and his colleagues engineered vascular sprouts in their iPSC-derived BBB model. They used ECs derived from the same patient's iPSCs to create vascular sprouts within the BBB. Unfortunately, they could not achieve the desired levels of perfusion via these sprouts [125]. In another attempt to achieve the vascularization, Cakier et al. used ESCs for their cerebral organoid model in which the ETS variant 2 is capable of reprogramming the fibroblasts into ECs, thus leading to vascularization. However, here the vascularization was observed only on the surface [126].

The microfabrication of organ-on-chips is an expensive process and requires special facilities like clean rooms, qualified fabrication engineers, yet it has limitations when it comes to modifying the chip. 3D printing techniques have overcome these limitations as it involves layer-by-layer printing of the desired tissue via the computer using hydrogel bioink. It can constitute of gelatin, hyaluronic acid, or alginate. These bioinks can be used for creating numerous cell types like astrocytes, neurons, etc. [3]. BBB models of OOC for glioblastoma have been generated using 3D printing that included vascular compartment structures to study the effect of drugs on tumour progression [127]. Using the two-photon lithography, synthetic scaffolds have been made to co-culture the neurons and the ECs. The microfluidics and 3D printing are different in terms of self-assembly and maturation of tissue [3].

24.5 Importance of Developing In Vitro Models of Human BBB

Several BBB models have been established and described in terms of barrier integrity and tightness, as well as the production of BBB-specific proteins and their application in physiology and pharmacology research [129]. Transwell systems using immortalized endothelial cells have always failed to produce tight barriers and have poor TEER values. Because lower molecular weight markers would pass through relatively fast, higher molecular weight markers like FITC dextran were frequently utilized in such systems. As previously stated, animal models may not always accurately represent human BBB function or a disease state (including

pathological characteristics such as onset, progression, etc.). Interspecies differences are unavoidable and are likely to play a significant effect. Terasaki et al., for example, used QTAP technology to establish that the mouse BBB is distinct from the human BBB [130]. Further, intraspecies differences might have an influence on data repeatability, affecting the results' translational value [131]. In vitro models based on immortalized human brain endothelial cells in static or dynamic settings have also been presented. These models reproduce at least some of the predicted properties of the BBB, such as high levels of expression of BBB-expressed receptors and transporters. As a result, "humanising" in vitro BBB systems using human derived cells may help to overcome current models' translational constraints and give a complimentary tool to existing in vivo techniques. The availability of human brain tissues for cell isolation is, however, one of the key limiting variables in reproducing a "humanised" in vitro model to research neurological illness or disease status. This is extremely difficult to come by, and most of it comes from post-mortem specimens (including foetal tissue) or surgical resections performed on patients [132, 133].

Although this may not reflect actual conditions in the original state, it is a good starting point. Even though the majority of in vitro BBB models now available are based on primary cultures of cerebral endothelial cells, endothelial progenitor cells (EPCs) could be another option for presenting a particular phenotype of EC. These methods use cord blood-derived hematopoietic stem cells to create a repeatable and stable human BBB model, in which the cells are first differentiated into endothelial cells (ECs). Following the differentiation phase, these ECs are co-cultured with pericytes to induce BBB characteristics [134, 135]. These models were discovered to create tighter barriers that can match the integrity of the human BBB. Nonetheless, due to diverse model designs and quantitative techniques, there are still hurdles ahead for creating the in vitro model of BBB. So even though cell culture-based in vitro models are useful for studying regulatory mechanisms modulating BBB physiology and function, as well as assessing the passage and transport mechanisms of putative brain penetrating drugs, reproducing the BBB properties in their entirety remains a significant and unsolved challenge [136]. Static and dynamic (flow-capable) systems, as well as the utilization of various cell types such as primary cells, immortalized cell lines, and, more recently, stem cells, have all been utilized to imitate the BBB in vitro. Cell cultures for BBB modelling have evolved in structural complexity, ranging from simple monocultures to multiple culture systems such as co-culture and tri-culture settings [24, 27].

The transwell system is one of the most widely used in vitro BBB models. Despite being highly user-friendly and reasonably simple to set up (Transwells also offer modest scalability and high throughput screening-HTS-capabilities), these systems have significant limits that must be considered. The two-dimensional structure, the lack of allowing endothelium exposure to shear stress, and "edge effects," where sections of the transwell walls bordering the membrane are innately extremely permeable, are all examples [133].

Subsequent technical advancements were subsequently established to allow the BBB endothelium to be exposed to physiological shear stress (SS), which modifies

not only endothelial morphology but also their function and physiological responses, opening the way for the development of "Dynamic Models". Synthetic hollow fibre structures made of thermoplastic polymers like polysulfone, polypropylene, and others were first used to construct brain microvessels and other CNS vascular beds [137], where EC and glia co-culture could be arranged to mimic the spatial and topographical distribution of these cells in situ, similar to the anatomy of brain microvessels. ECs acquire more stringent BBB features than static platforms under these regulated hemodynamic settings paired with exposure to glial cells, including high TEER, cell polarization, and expression of specialized transporters and efflux systems [45, 138]. Nevertheless, the excitement for the extra benefits provided by this dynamic in vitro-BBB (DIV-BBB) platform is limited by further design and construction flaws. There is no practical means to monitor the cells cultivated on or within the artificial microvessels since the concept depends on the usage of capillary-like tube structures enclosed by a bigger enclosure. When compared to appropriate brain microvessels, the capillaries' comparatively large diameter was more reflective of bigger vascular beds such as distal pre- and post-capillary segments. The DIV-BBB platform required a significant volume of chemicals and a large number of cells ($>10^6$) for culture start, which increased the cost, and the model did not have high throughput screening (HTS) capabilities. Additionally, unlike typical platforms (e.g. Transwells), the system configuration was highly difficult, requiring a large amount of time, money, and technical expertise [36, 46]. Furthermore, the most active and realistic in vitro BBB model, microfluidic devices, necessitates specialized equipment and technical abilities that are generally exclusive to the lab where the platform was established. Unfortunately, these constraints restrict the acceptance and development of these models in fundamental and translational research [136]. In silico models for assessing structure–activity connections for BBB penetration of pharmacological molecules based on their physicochemical characteristics have also been established. Although these models are less expensive, time demanding, and with high throughput screening approaches for new compounds in the drug development process, in vivo investigations must be used to verify the results [44].

Till date, various innovative BBB devices have been developed each with the aim to decipher the BBB physiology and thus opening new frontiers for the patho-physiologically relevant studies using these models (Table 24.1). These models closely recapitulate the micro-architecture of the brain and the complementary biochemical and mechanical microenvironments of the brain which are comparable to the results obtained via the in vitro experiments. The recent advancements in the BBB modelling foster a wider range of applications like studies related to alternative to animal-based studies, neuroinflammation, neurotoxicity, drug screening, disease modelling, etc. discussed briefly below.

Table 24.1 Microfluidics-based various models developed for the blood brain barrier studies

Year	Material used	Application	Reference
2012	PDMS	Assessing the permeability of the BBB	[46]
	PDMS	Influence of H_2O_2 and drug permeability	[139]
2013	PDMS	Assessing the permeability of BBB	[53]
2014	PDMS	Measurement of the tumour-cell migration	[140]
2015	PDMS	In vitro neonatal BBB on a chip	[54]
	PDMS	Cell-to-cell communication between endothelial cells, astrocytes, and pericytes and sampling of effluent from both sides of the barrier	[49]
2016	PDMS	Human neurovascular function and inflammation in vitro, and to identify physiological contributions of individual cell types	[119]
	PDMS	Use of pulsed electric fields to increase drug transport across the BBB through the transcellular pathway	[141]
	PDMS	Cytotoxicity and drug permeability of *Sunitinib*	[142]
2017	PDMS	Mechanical stimuli exerted by blood flow mediate both the permeability of the endothelial barrier and waste transport along the basement membrane	[143]
	PDMS	3D neurovascular microfluidic model consisting of primary rat astrocytes and neurons together with human cerebral microvascular endothelial cells for quantitative assessment of neuronal responses	[55]
	Photopolymer	In vitro drug permeability studies under recirculating perfusion	[144]
2018	Glass slide	Elucidating the regulation and relative contribution of PKCδ in the control of individual steps in neuroinflammation during sepsis.	[145]
	PDMS	In vitro approach for probing transport, efficacy, mechanism of action, and toxicity of neuroactive drugs	[51]
	Organoplate	Assessing the passage of large biopharmaceuticals, such as therapeutic antibodies, across the BBB	[146]
2018	PDMS/ PMMA	Multisite BBB chip to be used for screening drug, predicting their permeability through BBB as well as their toxicity.	[147]
	Organoplate	Potential utility of a membrane-free tetra-cultured brain-on-chip that can be scaled to high throughput as a cost-effective alternative method to animal testing	[148]
	Bio-hybrid	In vitro model for the investigation of BBB crossing of nanomaterials and drugs, envisaging therapeutic and diagnostic applications for several brain pathologies, including brain cancer	[117]
2019	Synvivo	Development of a 3D human BBB microfluidic model using human cerebral microvascular endothelial cells (hCMEC/D3) and primary human astrocytes within a commercially available microfluidic platform	[149]
	PDMS	Brain metastasis	[150]

(continued)

Table 24.1 (continued)

Year	Material used	Application	Reference
	PDMS	In vitro tool for development and validation of delivery systems that transport drugs and therapeutic antibodies across the human BBB	[99]
	PDMS	Human iPSC-derived BBB chip for disease modelling and personalized medicine	[109]
2020	PDMS	Effect of SARS-CoV-2 spike protein could have on brain endothelial cells	[151]
	PDMS	Mechanisms of transcytosis across the brain microvessel-like barrier of fluorescently tagged biologics, viruses, or nanoparticles	[152]
	PDMS	3D nanoparticle distributions at cellular levels and demonstrates the distinct cellular uptakes and BBB penetrations through receptor-mediated transcytosis	[153]

24.6 In Vivo and In Vitro Models: Alternatives to Animal Use

In vivo approaches have consistently supplied the most accurate data in BBB studies, and till date considered the gold standard [154]. Drugs are typically examined on animals before being studied in people in the pharmaceutical business. The effects of medications or therapies may be tracked at the cellular, tissue, organ, and systemic levels using these models. Animal models also allow researchers to investigate pharmacodynamics and pharmacokinetics and in immunological response. Animal models have the benefit of being able to depict the complexity of the BBB environment as well as individual variability found in humans [155]. In vivo investigations on animals, on the other hand, are expensive, time-consuming, and morally fraught [156]. Furthermore, translating animal models to human clinics is challenging, as illustrated by the fact that more than 80% of potential medications that passed animal model testing failed in clinical trials [157, 158]. This is due to a combination of factors, including flawed methodology and regulation of (some) animal experiments, as well as insufficient reproduction of human pathophysiology by genetically modified animals and species-to-species differences in expression profiles of, for example, transporter proteins [159].

In vitro cell and tissue models are quite often used as an alternate to animal experimentation and have developed over the last few decades [36]. These models are often made out of cells that have been cultivated in a controlled environment, making them more resilient, repeatable, and easier to evaluate than animal experiments [160]. These models, on the other hand, are frequently too simplistic to solve complicated research issues. The cell culture of brain-derived ECs can prove to be beneficial for assessing cytotoxicity of drugs, but are unsuitable for carrying out studies related to transport of drug across the BBB. The advancements in the culture systems have been developed to facilitate drug transportation research, like the Transwell configuration [161]. The Transwell culture technique is currently a

popular in vitro segmented culturing platform. It serves as a platform for drug research and permits co-culture of endothelial cells and other NVU-related cells [162]. Furthermore, human cells may be employed in these models, avoiding the challenges that in vivo animal models have when it comes to translating data to the clinic. However, these basic cultures frequently fail to duplicate crucial aspects of the BBB, such as shear stress and the NVU, making their prognostic relevance for human responses dubious [160]. In conclusion, in vivo animal models are widely praised because they allow researchers to investigate cellular, tissue, organ, and systemic functioning as well as pharmacodynamics and pharmacokinetics in a complex organism. They are, however, expensive, time-consuming, morally problematic, and frequently lack predictive usefulness. In vitro models, on the other hand, are more robust, repeatable, easier to analyse, and appropriate for high throughput than animal models, and they allow researchers to examine human cells and tissues. They are, however, frequently too basic to respond to sophisticated research problems.

24.6.1 Brain Tumour Studies

Glioblastoma multiforme (GBM) is one of the deadliest primary brain tumours, having 12- to 15-month median survival rate [163]. Meagre therapeutic results with high tumour recurrence rates following surgery, radiation, and chemotherapy are due to a lack of effective pharmacological therapies and a lack of knowledge of disease processes. Using BBB models, researchers investigated the interactions between glioma initiating cells and blood vessels, which play an important role in the invasion-ability of brain tumour cells, as well as the mechanism of pseudopalisade formation, which revealed that thrombosis can induce the migration of malignant glial tumour cells [164, 165]. Furthermore, novel BBB models for metastatic brain malignancies might help us better understand the pathways underlying tumour cells that spread to the brain. For instance, using only a BBB model, it was shown that migrating lung, breast, and melanoma cancer cells may damage BBB integrity, but not liver cancer cells [56]. Further progress in the construction of BBB models for GBM research aims to imitate the physiological structure of the BBB with even greater accuracy. For example, when ECs and GBM cells are co-cultured on a 3D porous microtubular scaffold, the basal lamina of the BBB is replicated, allowing cells to preserve their shape and phenotype [117]. As a result of this method, a hybrid microfluidic/spheroid model of patient-specific GBM was developed to test medication combinations with enhanced tumour-killing capability. Furthermore, microfluidic devices have been used to include 3D GBM spheroids of patient-specific ex vivo tissues [127, 166]. Yi and colleagues recently produced a bio-printed GBM-on-chip using patient-derived tumour cells, ECs, and decellularized ECM generated from brain tissue. We may expect future attempts to mimic the structural, biochemical, and biophysical features of the original tumour microenvironment to include the benefits of organoid research and microfluidic technologies, if this trend continues [127].

24.6.2 Identification of Neurotoxic Compounds

Screening and verification of neurotoxic chemicals are critical from a regulatory standpoint to assure the safety of consumer products [167]. Neurotoxic chemicals can cause a transitory or irreversible breakdown of the BBB in general [168]. Traditional animal regulatory studies for developmental neurotoxicity employ about 1400 animals and cost about US $1.4 million per chemical, but they still only allow for superficial testing of a few CNS functions [157]. Emerging BBB models could be a viable and less expensive way to assess xenobiotic-induced neurotoxicity, with the added benefit of incorporating human cells and producing more meaningful results. In a tetra-cultured BBB model composed of ECs, astrocytes, neuroblastoma, and microglial cells, organophosphate-based compounds (e.g. dimethyl methylphosphonate, diethyl methylphosphonate, diethyl cyanophosphonate, and diethyl chlorophosphate) were shown to penetrate the BBB and inhibit acetylcholinesterase activity. Toxicity levels determined on OOCs were found to be highly correlated with results acquired in vivo. Furthermore, methamphetamine, a neuroactive substance, was shown to cause reversible breakdown of the BBB in a BBB model. Proteomic and transcriptome investigations corroborated these findings, which were then verified in vivo [51]. A brain organoid-on-chip was built in another study to show that nicotine exposure disturbed cortical development and resulted in aberrant neuronal differentiation [169]. The benefits of microfluidic technology like in this unique arrangement is that the 3D co-culture and continuous perfusion were combined with organoid studies such as well-defined neuronal differentiation, regionalization, and cortical structure. Organoid-on-chip technologies appear to be a potential technique to study the human BBB, according to mounting data [52].

24.6.3 Deciphering the Link Between Inflammation and Neurodegeneration

The activation of microglia and astrocytes causes neuroinflammation, which is a critical component of neurodegenerative diseases. When glial cells are activated for a long time, inflammatory cytokines, including tumour necrosis factors and interleukins, are released [170]. Immune cells invade the brain in an inflammatory state and in neurodegenerative disorders, traversing the BBB breakdown site and causing irreparable brain damage [171]. Immune cells play a crucial function in tissue healing, but if they are present in brain tissue, they might cause injury by generating damaging reactive oxygen species [172]. Several BBB models have been developed to date to study neutrophil infiltration into extravascular tissue and to aid in the identification of therapeutic strategies to reduce neutrophil adhesion and migration over time, and thus neuronal degeneration [173]. TNF-induced neuroinflammation, for example, was demonstrated ‘on-chip’ to promote the release of numerous inflammatory cytokines and lower the expression of ZO-1 proteins. Under ischemia-like circumstances (i.e. oxygen-glucose deprivation), the integrity of the BBB is disturbed, resulting in neutrophil transmigration across the BBB. The

administration of IL-8, a strong neutrophil activator and chemoattractant, on the other hand, does not cause neutrophils to cross the BBB. These findings show that an intact BBB inhibits neutrophil infiltration into the brain, and that the BBB model is appropriate for observing BBB damage in pathological situations [174]. Furthermore, a time-dependent association between cytokine activation and loss of barrier function (expressed in terms of changed metabolomic signature patterns) has been revealed in an elegant BBB model capable of metabolite analysis to uncover important pathways in inflammatory reactions. Protein kinase C-delta has also been shown to have a role in modulating neutrophil transmigration across the BBB [175]. Current microfluidic models for Alzheimer's disease, Parkinson's disease, and neuroimmune responses, however, do not incorporate a BBB [52].

24.6.4 Drug Development

The in vitro cell culture approach is significantly more effective at assessing diseases that undermine the integrity of a single organ, but it falls short when it comes to diseases that affect numerous organs simultaneously. Diseases such as hepatic encephalopathy, cancer cell metastasis in brain tissues, brain-gut axis-mediated CNS disorders, immune-related diseases mediated by the brain-spleen axis, and muscular lesions producing CNS injury, will be difficult to investigate using in vitro cell culture systems [176]. The brain metastasis of malignancies and its processes have not been investigated due to a lack of efficient in vitro platforms. A lung-brain-chip model was created for the first time to investigate the processes of non-small cell lung cancer brain metastasis (NSCLC). Human lung cancer cell line (PC9), bronchial epithelial cells (16HBE), pulmonary microvascular endothelial cells and fibroblasts (HFL1), and human mononuclear cells (THP-1) are all mixed on a chip, together with human astrocytes and BMECs [52]. Furthermore, via modulating the MEK/ERK signalling pathway, Aldo-keto reductase family 1 B10 has been shown to contribute to in situ brain metastases of non-small cell lung cancer (NSCLC) [177]. This effective microfluidic lung-brain microchip simulation aids in the rapid presentation of the dynamic process of NSCLC brain metastasis as well as the creation of therapeutic medications. An increasing amount of evidence shows that the microbiota-gut-brain axis has a role in the development of neurological illnesses such as Alzheimer's, Parkinson's, depressive symptoms, and cerebral stroke. A range of ethnic medications have been shown to boost the microbial population in the intestine, which may help to treat neurological illnesses indirectly [52]. Nonetheless, we presently need a solid framework for investigating gut-brain inter-organ interactions. It's still a question whether nerve conduction or chemical transmitters connect the two distant organs of the human body. The created microfluidic brain-gut chip technology, on the other hand, has the potential to answer the puzzle of intestinal-brain connection. It will allow researchers to investigate the microbiota-gut-brain axis' neuroelectrical signal transduction as well as a range of neurohumoral variables involved [163, 164]. In vitro chip models are also absent for hepatic encephalopathy, the microbiota-gut-liver-brain axis, and encephalopathy-

related muscular discomfort and atrophy. While brain-related organ-on-chip platforms have a lot of potential, they are still in their infancy and require further research and testing [52].

Most BBB chips can only imitate the basic fluid channel topology of the brain vascular network at the moment, which is a challenge that must be overlooked. The simulation of dynamic hemodynamics appears to be incompatible with the real BBB multi-stage complicated vascular network. The novel leaf-chip concept appears to have the potential to miniaturize the vascular network building of BBB or other brain-related organ illnesses [127]. In fact, a never-ending flood of single-organ microfluidic chips is developing, including BBB. We must accept the arrival of BBB-related multi-organ chips without reservation.

24.6.5 Drug Screening and Efficacy Evaluation

In vitro BBB models with high fidelity can also help with early screening of medication candidates for the brain, such as new biopharmaceuticals and nanomedicines. In this vein, a high-throughput BBB model (with 6–12 units) has been utilized to find compounds that pass the BBB in preliminary drug permeability tests [144]. The BBB system was used to assess permeability coefficients for model pharmaceuticals (e.g. caffeine, cimetidine, and doxorubicin), which demonstrated high correlation with in vivo results. Other HTS BBB models include two 16-unit devices that are used to compare the efficacy of chemotherapeutics commonly used in clinical settings (e.g. lipophilic temozolomide versus hydrophilic gemcitabine), or to compute the TEER values with an integrated multi-electrode array, allowing for a label-free real-time analysis of barrier function [52]. Substantial biopharmaceuticals, such as monoclonal drugs, have also been evaluated to see if they can penetrate the BBB, which is frequently a difficult task that prevents efficient distribution into the brain [146].

Nanomaterials, on the other hand, are promising medication carriers. The convoluted cerebrovascular supply network, as well as the delicate physiological and anatomical structure of one of the most complex organs in the human body, provide challenges when investigating CNS illnesses in in vitro models. In vitro microfluidic BBB chips have recently emerged as a lighthouse and guidepost for brain science research in the twenty-first century [50, 52]. Primarily, the multi-cell co-culture technique has the potential to be used to investigate CNS illnesses. Human BMECs line HCMEC/D3 were used to test the effectiveness of sunitinib traversing the BBB on a 3D chip [178]. Yu's team has created a pump-free tricellular BBB chip. TNF stimulation and dexamethasone therapy were demonstrated to be efficacious in this system using co-cultured primary BMECs, pericytes, and astrocytes from neonatal rats. Trans-epithelial electrical resistance (TEER) has also been effectively assessed as a marker of BBB barrier function. On polydimethylsiloxane, HCMEC/D3, primary rat brain endothelial cells, pericytes, and astroglia cells were co-cultured (PDMS). An indium tin oxide electrode was used to record the TEER [56]. Likewise, SD rats' cortical astrocytes, pericytes, and neural stem cells were co-cultured with mouse BMECs line bend.3 on a 3D bio printed microvascular

network system. This in vitro BBB model, which was created using four diverse types of cells, has a higher TEER and a tighter intercellular link. Furthermore, FITC-labelled Angio2, TAT, and hoxa-13 small molecular polypeptides demonstrated excellent permeability in this experimental system [159]. However, the variances, compatibility, and mutual exclusion among species in these BBB systems generated by co-culture of cells from diverse species and genera need additional research. More promisingly, four kinds of human cells were co-cultured to create an in vitro BBB chip model, including BMECs generated by neural stem cells from primary foetal brain tissue, and primary foetal cerebral cortex astrocytes and primary pericytes. This model further proved the efficiency of penetration on diazepam, caffeine, ibuprofen, celecoxib, diclofenac, loratadine, and rhodamine 123 [58].

Second, studying the functioning of synapses in subcellular structures presents several problems. Changes in presynaptic neuron activity, such as Ca^{2+} ions concentration, cause neurotransmitters to be released in the synaptic cleft, leading postsynaptic neurons to respond to the stimulus signal. However, how can the transmission channel of electrical impulses and the biochemical regulating medium of sensory neurons be properly and in real time recorded? Multiple microelectrodes and a suitable microimaging system were used to record the synaptic activity and dynamic processes of rat primary cortical neurons on a PDMS chip [59]. On a BBB chip, this approach based on microfluidics and numerous microelectrodes allows researchers to examine subcellular features and quantify the electrical activity of neurons. The physiological barrier function of a 3D-BBB chip made from iPSC-derived BMECs has been demonstrated in studies [119, 143]. A more complicated BBB chip that included iPSC-derived BMECs, human primary astrocytes, and vascular pericytes similarly demonstrated substantial barrier function and electrical conduction features. This can be used to assess the effectiveness of BBB-penetrating medicines and antibodies in hypoxic encephalopathy and other CNS illnesses. A BBB chip made up of the three types of cells mentioned above was used to perform precision screening of specific disease indicators as well as assessment of neuroprotective medication actions [179–181].

Third, if the medicine is unable to reach or is less concentrated in the sick area of the brain, it will have a poor therapeutic impact. As a result, methods to ease drug transport across the BBB hold a lot of promise. The human BMEC-built free-filtering BBB chip exhibited effective filtration of dextrans, low-density lipoprotein, and PEG-P (CL-g-TMC) polymersomes of various molecular sizes [182]. Due to receptor-mediated transcytosis, this allows the researchers to measure the efficiency of nano-drugs passing across the BBB in a quantifiable manner. The above-mentioned BBB chip is also utilized to test the penetrability of rhodamine-labelled polyurethane nano drug medium via the BBB [147].

Additionally, drug carrier carriers might be regarded as an option. On a human BMECs imitatively infected BBB chip, L-glutathione-conjugated poly (ethylene imine) has demonstrated exceptional performance as an antioxidant in assisting drug penetration through BBB. It also has no cytotoxicity in mouse fibroblast L929 and human embryonic kidney HEK-293 cells, as well as no blood system side effects [183]. Biocompatible and biodegradable nanoscale drug carrier raw

materials, on the other hand, are extremely useful and optional. Among these, the effect of nanoparticle shape and surface roughness on drug adherence to vascular endothelial cells should be considered. Elongated rod-shaped nanoparticles and smooth surfaces are more likely to prevent medications from adherence to vascular endothelial cells than spherical nanoparticles and rough surfaces. As a consequence, the impact of hemodynamics on drug-vessel adhesion can be bypassed or minimized, allowing medications to have a greater therapeutic effect on CNS illnesses, as indicated by reducing the violent impact of blood flow [184]. Apart from its use in the manufacture of BBB chips, microfluidic also has a bright future in the creation of drug-loaded nanoparticles. Anti-HIV medications have a tough time passing across the BBB to exert their effects since the BBB is one of the key targets for the human immunodeficiency virus (HIV). The micro fluidization-based and poly (lactic-co-glycolic) acid-loaded efavirenz nanoparticles provide a number of benefits over traditional approaches. It demonstrates greater tissue dispersion with a smaller size, a lower absolute zeta-potential, higher drug loading, and a longer drug effectiveness maintenance period [185].

Fourth, electrochemical approaches may be more convenient and quicker to test the effectiveness of CNS medicines using microfluidic BBB chips than traditional molecular biology procedures. The use of an electrochemical approach to measure permeability in 3D-cultured human umbilical vein endothelial cells (hUVECs) eliminates the need of bulky and time-consuming fluorescent-tagged permeability-related proteins [45]. TEER of pulmonary and intestinal chips made by primary human airway epithelial cells and Caco-2 cells, respectively, was also measured using integrated electrodes tests [186]. Similarly, TNF- and isoproterenol stimulation of vascular permeability measurement and cardiac function evaluation was performed concurrently using hUVECs and human iPSC-derived cardiomyocytes in a heart-on-a-chip. Furthermore, the scientists used impedance spectroscopy and electrical modelling to explore the intestinal barrier function and villi development features in a Caco-2 cell-based intestinal chip [52].

It may also be used to evaluate the barrier performance of a BBB chip in vitro and to record the process of tissue development in real time. The aforementioned microfluidic BBB chips are made up of 2–4 several types of cells, with stem cell orientated differentiation cells and/or primary neurons being the most prevalent. However, the unpredictability and diversity of stem cell development, as well as the difficulty in collecting primary cells, are limits that must be considered and optimized further [135]. On the other hand, and more critically from the standpoint of clinical conversion, we always propose using human cells. Finally, to investigate medication effectiveness on microfluidic BBB chips, minimally invasive electrochemical approaches and cell type selection should be considered.

24.6.6 Personalized Medicine

The advancement of stem cell-based technologies for personalized medicine is reaching a pivotal point in its development. Individual patients'

neuro-pathophysiology and treatment response to different illness kinds might vary substantially since they have diverse genotypical and phenotypic traits [187]. ECs, neurons, microglia-like cells, and pericytes-like cells have all been produced from brain-derived human induced pluripotent stem cells (hiPSCs) in both healthy and sick people [93]. When employing hiPSC-derived neurons to simulate specific brain illnesses, however, it's important to remember that different brain areas have varied cell composition, protein expression, metabolism, and electrical activity patterns. Due to these issues, a multiregional brain-on-chip model was developed; however, it did not incorporate a BBB [188]. Similar to the basic BBB in embryos and the spinal cord in adults, an organ-on-chip containing spinal-derived motor neurons and BMECS from hiPSCs has been produced [189]. Till date, the only BBB device described that used hiPSC-ECs had a TEER value that was comparable to in vivo and much higher than previous BBB models. In the near future, hiPSC-derived pericytes, astrocytes, BMECs, and neurons might be included into BBB models, resulting in a fully isogenic model or 'patient-customized' approach. As a result, medicine dose and potential side effects might be evaluated for individual patients, and BBBs for uncommon diseases could be created to investigate specific pathologies when animal models are lacking [52].

24.6.7 Viral Infection Studies

The BBB is an important aspect of the immune system's defence against CNS viral infections like *Flaviviridae* (e.g. Zika virus, encephalitis virus, and West Nile virus). There are no particular targeted therapies available at this time, and survivors of severe infections are frequently left with long-term neurological impairment, such as paresis congenital infection and its consequences. The modification of the BBB during neuroinflammation and neuronal death is a characteristic of neurotrophic viral infections. BBB integrity has been shown to be disrupted in flavivirus-infected mouse models [190, 191]. However, the specific process by which viruses reach the CNS remains a mystery. One idea is that microvascular ECs are directly infected by transcellular uptake. The virus was able to penetrate the BBB without affecting the integrity of the endothelium monolayer in a transwell BBB model. Yet, the specific process by which viruses reach the CNS remains a mystery. One idea is that microvascular ECs are directly infected by transcellular uptake. The virus was able to penetrate the BBB without affecting the integrity of the endothelium monolayer in a transwell BBB model. However, the utilization of mono-cell culture and the lack of physiological TEER values are two major drawbacks of the transwell technique. The use of BBB models in the study of neurotropic viral infections might give a more realistic picture of the infection process and accelerate treatment development [52].

24.7 Regulations Concerning Translational Acceptance of OOC

OOAC is a game-changing technology with the potential to transform the efficiency, efficacy, and prices of drug development; to enhance human biology understanding; and to facilitate clinical research when human trials are not possible. Furthermore, research and development are required for this technology's effective implementation and acceptance. Technological maturity, more rigorous validation of translational and predictive in vivo-like biology, and need for tighter quality standards for commercial viability are all areas for improvement. Scientists blended fluidic systems, cell culture techniques, analytical methodologies, and single, 2D, and 3D cell culture protocols into novel in vitro models around 10 years ago, resulting in the OOACs idea. OOAC systems were created to mimic the functioning of human organs in microliter quantities. The promise of these models, also known as "microphysiological systems," drew the attention of researchers and pharmaceutical corporations searching for more effective, efficient, and cost-effective ways to decrease drug development failures. Materials, biological samples, and interaction with other technologies are all part of an OOAC system, which is a novel interdisciplinary idea. It has the potential to become a vital component of the drug testing and drug screening process, which is a highly regulated industry, based on its definition. The goal of the study was to learn about consumers' levels of knowledge and established firms' approaches to applicable categories and legislation. To our knowledge, the primary regulatory organizations, such as the US Food and Drug Administration (US FDA), the European Medicines Agency, and/or the Medicines and Healthcare Products Regulatory Agency in the United Kingdom, have not classified OOAC as a particular device.

Nevertheless, the FDA's Office of Clinical Pharmacology, Centre for Drug Evaluation and Research (CDER), and Division of Applied Regulatory Science began reporting their first evaluation and co-development of OOAC (micro engineered human cellular systems, microphysiological systems) as new revolutionary models to improve the assessment of drug safety and the general understanding of diseases caused by bacteria and chemicals in their annual reports in 2017 [192]. Many extant quality management and regulatory systems may be relevant to OOAC manufacturing and validation, and the OOAC community might use them until new standards are developed. A globally agreed-upon quality management system has been developed and is updated on a regular basis by the International Organization for Standardization (ISO 9001:2015). By defining requirements and guidelines for documenting information, process planning, leadership and resource management, performance evaluations, and preventive/corrective actions, this system can be used by any type of organization to demonstrate the ability to consistently provide high-quality products and services. ISO 13485 is a quality management system similar to ISO 9001, but it focuses on requirements specific to the medical device industry, such as design and development planning, inputs, outputs, review, verification, validation, transfer, and control; cleanliness, contamination control, and sterility; monitoring, audits, and traceability, to name a few [192]. Clinical laboratory improvement amendments (CLIA) released by the US Centres for Medicare and

Medicaid Services for clinical diagnostics or personalized medicine may be relevant to OOAC models. CLIA laws apply to establishments that provide human diagnostic, preventive, or disease treatment services, as well as health assessments. While the specific certification criteria vary based on the type of lab test performed and its complexity, they often involve proficiency testing, human competency assessments, quality assessments, as well as maintenance, quality checks, calibrations, and corrective action processes.

The US Food and Drug Administration (FDA) has produced Good Laboratory Practice for on-clinical Laboratory Research (21 CFR Part 58), which covers preclinical toxicity studies [192]. The regulations call for the establishment of a quality assurance unit, a study director, and specific testing facility management, as well as specific requirements for specimen and data storage, study protocols and standard operating procedures (including corrective actions), reagent and solution tracking (including batch numbers and expiration dates), written records for equipment maintenance, cleaning, calibration, and design, and the handling and archival of samples from each. The FDA also created a Qualification of Drug Development Tools Qualification Program (FD&C Act Section 507), which includes the Animal Model Qualification Program (AMQP) and the Biomarker Qualification Program (BQP), both of which might be used to OOACT. The AMQP certification assesses the model's suitability for a particular application, including characterization of the challenge agent(s) and exposure, main and secondary endpoints, intervention triggers, and critical illness values to be duplicated for quality assurance and control. In particular, our poll found that 44% of OOAC developers and 50% of end-users were unaware of any of the existing regulatory requirements and principles listed above.

The most often utilized standards among OOAC developers, according to our poll, are ISO 9001:2015 (22%), FDA 21 CFR Part 58 (22%), and FDA FD&C Act Section 507 (22%). Only 2% of OOAC developers questioned said they used ISO standard 13485 for medical devices, while CLIA standards (4%) and FDA AMQP (4%) were also low. Since none of the regulatory requirements and quality management techniques listed above are a perfect fit for OOAC technology, expertise with these frameworks would assure the reliability, repeatability, and completeness of the experiments and data created using OOAC models. It would be feasible to develop new and more accurate standards and rules by referring to shared protocols for data testing, collecting, and analysis. This higher degree of quality and regulatory assurance would have a favourable influence on the technology's acceptance in the scientific and industrial sectors, as well as the categorization of new OOAC goods on the market. Compliance with quality standards and certifications is not usually addressed in academic research, at the proof-of-concept stage or low TRLs, due to the expenses associated with reviewing and enhancing current processes, qualification of equipment, reference materials, and procedures, to mention a few. Compliance with quality and regulatory standards for method validation, risk and requirement management, documentation and traceability, personnel training, and inter-laboratory comparisons would, on the other hand, support robust and repeatable development and fabrication of OOAC devices and platforms for emerging

small businesses, and inevitably boost the adoption and establishment of OOAC models as the new standard for highly regulated end-users, such as the pharmaceutical industry. Existing standards for hardware components and procedures used in the OOAC area, such as the usage of analytical-grade tubing and high-pressure fluidic fittings from ISO-certified vendors, might be implemented in the lack of particular regulatory requirements. Learning to establish corrective and preventive measures (CAPA), as stated by the US Food and Drug Administration and the Medical Device Innovation Consortium in a recent white paper, might be advantageous as well.

24.8 Challenges Towards Development of OOCs

The development of BBOCs that can be widely available for BBB research still faces obstacles (Fig. 24.2). One of them is to provide widely agreed standards for quantitative evaluation of a BBB-on-chip model's functioning. A few major obstacles are discussed below, that must be considered while constructing and evaluating BBOCs, as well as elements on which researchers must agree.

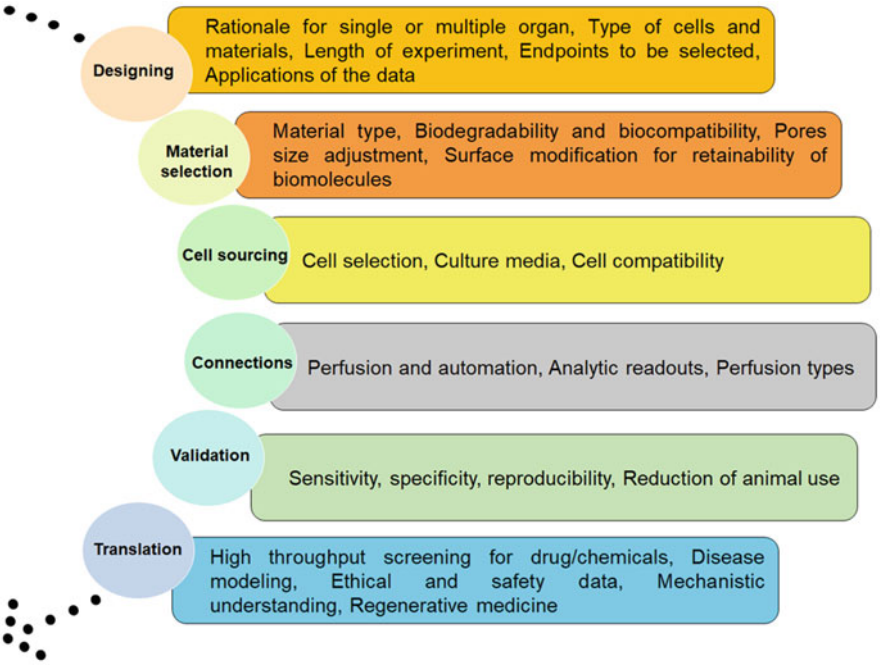

Fig. 24.2 Challenges towards the development of organ-on-a-chip model for the blood brain barrier (BBB): A blueprint of OOC challenges and requirements, starting with permeability considerations and progressing through development phases, including TEER, cellularization, perfusion and automation, and validation requirements, before reaching a translational stage that allows for specific regulations, standardization, and provides a level of ease-of-see that can lead to greater efficiency

24.8.1 Permeability

The BBB's primary role is to maintain brain homeostasis and, more specifically, to protect the brain from toxic compounds in the blood. The permeability of the cell barrier should be measured to assess the performance of BBOCs. If the permeability matches physiological values, then a useful BBB-on-chip has been created that may be used to evaluate drug candidates. The tight connections between endothelial cells physically prevent big and hydrophilic molecules, such as dextrans and ions, in general. Carriers and receptor-mediated transport actively transfer ions and vital nutrients such as glucose, amino acids, peptides, and hormones into the brain [159].

Tiny lipophilic molecules (400–500 Da) can pass across the BBB with little difficulty. Multidrug resistance transporters, on the other hand, control the efflux of potentially hazardous compounds, such as lipophilic drugs [193]. Analytes from all of these classes must be evaluated in the device to determine the whole barrier function: both hydrophilic and lipophilic molecules that are passively and actively transported or expelled. All of the currently available BBB devices exhibit the measurement of barrier permeability. However, this permeability must be quantified in a method that allows it to be compared to in vivo and other in vitro data. For passive transport, this may be estimated in vivo by computing the permeability coefficient of an analyte (cm/s), which is independent of the analyte concentration, flow rate, and device size. When measuring permeability in organs-on-chips, one issue that might emerge is the contribution of routes of transport other than diffusion (or active transport). Convective flow will occur via breaches in the cell barrier if there is a pressure differential between the two compartments, for example. Furthermore, if there is a differential in solute concentration between the channels, osmotic-driven flow can occur. These effects must be recognized and minimized as much as possible in permeability measurements, for example, by employing identical compartments with the same applied pressure and the same fluid (culture medium) on both sides of the barrier. The presence of astrocytes on the basal side of the membrane, for example, can also affect permeability measurements. These are said to constrict the BBB, although they will also function as a physical barrier to diffusion on their own. As a result, additional cells should constantly be checked for their contribution to the total barrier function. To summarize, the cell layer's permeability is a critical readout for BBOCs. Different analytes that are passively or actively transported, excreted, or metabolized should be evaluated using a proper procedure to establish physiological relevance. A common metric independent of the microdevice design, such as the permeability coefficient, should be supplied to allow comparison amongst platforms [159].

24.8.2 TEER

The transendothelial electrical resistance (TEER) is a common metric for determining barrier tightness [71, 194–196]. The electrical resistance to paracellular transport is largely represented by TEER: the tighter the cell layer is packed, the fewer holes in

the cell barrier exist through which ions and other charged species can pass, resulting in a greater resistance [195]. Ion transport through paracellular channels generated by tight junction proteins and transcellular transport of ions (through transporters) are only detected when the cell barrier is sufficiently strong and the contribution of paracellular ion transport routes is limited [197]. Measuring the TEER has the distinct benefit over the previously mentioned permeability measures in that it is a rapid, non-invasive, and label-free method of determining barrier tightness. Furthermore, if a proper electrode material and measuring method are selected, and the measurement electrodes are incorporated into a microfluidic BBB-on-chip system, real-time measurements may be done [198, 199]. To conclude, TEER is a highly important indication of barrier tightness that can be assessed swiftly, non-invasively, without the need of labels, and in real time. However, in order to arrive at dependable TEER readings and to be able to compare various platforms, the method by which the TEER is measured must be well thought out [159].

24.8.3 Cell Selection and Sourcing

The cells employed are crucial to the BBB-on- chip's physiological significance. The more closely the cells resemble the human BBB, the more accurate the model will be. Many BBB-on-chip models and other in vitro BBB models have previously relied on animal cells [100]. Because in vitro data can be more easily compared to in vivo results from the same species, using these cells can give useful information for validation. Human cells, on the other hand, would be the most predictive, and hence the cells of choice for future drug development [200]. As ECs from brain capillaries already have the right expression profile, they have been the first choice. Keeping this trait in vitro after numerous passages, however, has been difficult [41]. Brain-derived ECs are more widely available and have less batch-to-batch variation, but they also lost some of their phenotypic and genotype during the immortalization process. Deriving brain-specific ECs from human induced pluripotent stem cells, on the other hand, has progressed. Because brain endothelium may be produced from both healthy and diseased cells (e.g. with genetic abnormalities resulting in BBB pathology in vivo), as well as cells coming from various persons or groups, this breakthrough offers enormous potential for customized treatment [201]. As indicated in the introduction, additional cells from the NVU, such as astrocytes and pericytes, are necessary for the creation and maintenance of the barrier, in addition to endothelial cells. If these cell types are added, the model will be more physiologically realistic, and some current BBOCs have already shown an increase in barrier tightness when these cells are included. However, in the majority of these systems, the two cell types are cultivated in distinct channels or chambers, separated by a membrane of several micrometres in thickness. A thinner barrier that allows cell-cell contact or no membrane at all would be more physiological, but more difficult to construct and test consistently [44, 202].

Hydrogel-based devices, such as those developed by Kim and Cho are predicted to be useful for this purpose because they allow direct interaction between

endothelial cells lining the lumen and other cells cultivated in 3D in the surrounding gel [59, 174]. In summary, animal cells are commonly employed because they allow for simple comparison of in vitro and in vivo outcomes within the same species. Human cells in BBOCs, on the other hand, would be most useful for drug development studies or research of human BBB physiology and disease, despite the tissue source's restricted availability. Recent research suggests that generating brain endothelium from human iPSCs cells might be a more accessible source of relevant cells [159, 203].

24.9 Future Directions

The BBB has sparked a lot of curiosity because of its significance in both health and sickness as the brain's gatekeeper. This requires the development of adequate in vitro human models that faithfully duplicate its primary physical and functional features. Despite recent advances in the ability to engineer novel in vitro systems, major challenges remain, such as incorporating all relevant BBB cell types, using an appropriate 3D hydrogel to mimic the brain ECM, and achieving in vitro vascularization to emulate transport across this barrier, especially for therapeutics in the context of neurological diseases. Future efforts and advances in the vascularization of cerebral organoids, including all critical BBB cells in a brain-like matrix, will certainly result in platforms that accurately replicate the human BBB. More importantly, in order to capture changes in barrier integrity in health and sickness, permeability testing procedures must be improved. In vitro BBB models with varied transport properties will likely offer up new possibilities for modifying barrier function and properly recreating neurological disorders. Furthermore, linking in vitro brain models to other OOCs and perfusing the connected systems with patient plasma or blood would replicate upstream effects on the BBB from other organs or systemic effects on multi-OOCs from perfused blood. However, before these advanced physiological BBB models can be developed, brain cells must first be described, including their specific markers and genotypes. Existing methods rely mostly on in vitro characterization tools used to separate, sort, and cultivate cells taken from fresh brain tissue, where function and markers are inevitably lost. The development of new mapping methodologies for gene expression in vivo will allow the construction of adequate baseline values for the diverse cell types of the BBB.

References

1. Wu Q, Liu J, Wang X, Feng L, Wu J, Zhu X et al (2020) Organ-on-a-chip: recent breakthroughs and future prospects. Biomed Eng Online 19(1):9
2. Lagowala DA, Kwon S, Sidhaye VK, Kim DH (2021) Human microphysiological models of airway and alveolar epithelia. Am J Physiol Lung Cell Mol Physiol 321(6):L1072–L1L88
3. Hajal C, Le Roi B, Kamm RD, Maoz BM (2021) Biology and Models of the blood–brain barrier. Annu Rev Biomed Eng 23:359–384

4. Anaya EF (2020) Age-related alterations on ultrastructure and gene expression profile of the female blood-brain barrier. Open University, United Kingdom
5. Pinheiros LBP, Aversi-Ferreira TA (2021) Blood–brain barrier and the virus diseases. Dement Neuropsychol 15:419–420
6. Liebner S, Dijkhuizen RM, Reiss Y, Plate KH, Agalliu D, Constantin GJ (2018) Functional morphology of the blood–brain barrier in health and disease. Acta Neuropathol 135(3): 311–336
7. Li J, Li C, Yuan W, Wu J, Li J, Li Z et al (2017) Mild hypothermia alleviates brain oedema and blood-brain barrier disruption by attenuating tight junction and adherens junction breakdown in a swine model of cardiopulmonary resuscitation. PLoS One 12(3):e0174596
8. Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic BV (2019) Blood-brain barrier: from physiology to disease and back. Physiol Rev 99(1):21–78
9. Banks WA (2019) The blood-brain barrier as an endocrine tissue. Nat Rev Endocrinol 15(8): 444–455
10. Abbott NJ, Rönnbäck L, Hansson E (2006) Astrocyte–endothelial interactions at the blood–brain barrier. Nat Rev Neurosci 7(1):41–53
11. Huber JD, Egleton RD, Davis TP (2001) Molecular physiology and pathophysiology of tight junctions in the blood–brain barrier. Trends Neurosci 24(12):719–725
12. Archie SR, Al Shoyaib A, Cucullo L (2021) Blood-brain barrier dysfunction in cns disorders and putative therapeutic targets: an overview. Pharmaceutics 13(11):1779
13. Segarra M, Aburto MR, Acker-Palmer A (2021) Blood–brain barrier dynamics to maintain brain homeostasis. Trends Neurosci. https://doi.org/10.1016/j.tins.2020.12.002
14. Cai Z, Qiao P-F, Wan C-Q, Cai M, Zhou N-K, Li Q (2018) Role of blood-brain barrier in Alzheimer's disease. J Alzheimers Dis 63(4):1223–1234
15. Allgaier J, Lagu T, Haessler S, Imrey PB, Deshpande A, Guo N et al (2021) Risk factors, management, and outcomes of legionella pneumonia in a large, nationally representative sample. Chest 159(5):1782–1792
16. Li W, Qiu J, Li X-L, Aday S, Zhang J, Conley G et al (2021) BBB pathophysiology–independent delivery of siRNA in traumatic brain injury. Sci Adv 7(1):eabd6889
17. Abbott NJ (2013) Blood–brain barrier structure and function and the challenges for CNS drug delivery. J Inherit Metab Dis 36(3):437–449
18. Sivandzade F, Bhalerao A, Cucullo LJ (2019) Cerebrovascular and neurological disorders: protective role of NRF2. Int J Mol Sci 20(14):3433
19. Abdullahi W, Tripathi D, Ronaldson PT (2018) Blood-brain barrier dysfunction in ischemic stroke: targeting tight junctions and transporters for vascular protection. Am J Physiol Cell Physiol 315(3):C343–CC56
20. Obermeier B, Daneman R, Ransohoff RM (2013) Development, maintenance and disruption of the blood-brain barrier. Nat Med 19(12):1584–1596
21. Baeten KM, Akassoglou KJ (2011) Extracellular matrix and matrix receptors in blood–brain barrier formation and stroke. Dev Neurobiol 71(11):1018–1039
22. Xu L, Nirwane A, Yao YJS, Neurology V (2019) Basement membrane and blood–brain barrier. Stroke Vasc Neurol 4(2). https://doi.org/10.1136/svn-2018-000198
23. Lecuyer M-A, Kebir H, Prat A (2016) Glial influences on BBB functions and molecular players in immune cell trafficking. Biochim Biophys Acta 1862(3):472–482
24. Gomes MJ, Mendes B, Martins S, Sarmento B (2016) Cell-based in vitro models for studying blood–brain barrier (BBB) permeability. Concepts and models for drug permeability studies. Elsevier, Amsterdam, pp 169–188
25. Butt AM (1995) Effect of inflammatory agents on electrical resistance across the blood-brain barrier in pial microvessels of anaesthetized rats. Brain Res 696(1-2):145–150
26. Stamatovic SM, Johnson AM, Keep RF, Andjelkovic AV (2016) Junctional proteins of the blood-brain barrier: new insights into function and dysfunction. Tissue Barriers 4(1):e1154641

27. Kaisar MA, Sajja RK, Prasad S, Abhyankar VV, Liles T, Cucullo L (2017) New experimental models of the blood-brain barrier for CNS drug discovery. Expert Opin Drug Discov 12(1): 89–103
28. Galla H-J (2018) Monocultures of primary porcine brain capillary endothelial cells: still a functional in vitro model for the blood-brain-barrier. J Control Release 285:172–177
29. Alluri H, Shaji CA, Davis ML, Tharakan B, Injury I (2018) A mouse controlled cortical impact model of traumatic brain injury for studying blood–brain barrier dysfunctions. Methods Mol Biol 1717:37–52
30. Sivandzade F, Prasad S, Bhalerao A, Cucullo L (2019) NRF2 and NF-κB interplay in cerebrovascular and neurodegenerative disorders: molecular mechanisms and possible therapeutic approaches. Redox Biol 21:101059
31. Helms HC, Abbott NJ, Burek M, Cecchelli R, Couraud P-O, Deli MA et al (2016) In vitro models of the blood–brain barrier: an overview of commonly used brain endothelial cell culture models and guidelines for their use. J Cereb Blood Flow Metab 36(5):862–890
32. Pardridge WM (2012) Drug transport across the blood–brain barrier. J Cereb Blood Flow Metab 32(11):1959–1972
33. Agoston DV (2015) Bench-to-bedside and bedside back to the bench; seeking a better understanding of the acute pathophysiological process in severe traumatic brain injury. Front Neurosci 6:47
34. Weiss N, Miller F, Cazaubon S, Couraud P-O (2009) The blood-brain barrier in brain homeostasis and neurological diseases. Biochim Biophys Acta 1788(4):842–857
35. Organization WH (2006) Neurological disorders: public health challenges. World Health Organization, Geneva
36. Naik P, Cucullo L (2012) In vitro blood–brain barrier models: current and perspective technologies. J Pharm Sci 101(4):1337–1354
37. Wong A, Ye M, Levy A, Rothstein J, Bergles D, Searson PC (2013) The blood-brain barrier: an engineering perspective. Front Neuroeng 6:7
38. Terstappen GC, Meyer AH, Bell RD, Zhang W (2021) Strategies for delivering therapeutics across the blood–brain barrier. Nat Rev Drug Discov 20(5):362–383
39. Westein E, van der Meer AD, Kuijpers MJ, Frimat J-P, van den Berg A, JWM H (2013) Atherosclerotic geometries exacerbate pathological thrombus formation poststenosis in a von Willebrand factor-dependent manner. Proc Natl Acad Sci U S A 110(4):1357–1362
40. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE (2010) Reconstituting organ-level lung functions on a chip. Science 328(5986):1662–1668
41. Abbott N, Dolman D, Yusof S, Reichel A, Hammarlund-Udenaes M, de Lange E et al (2014) Drug delivery to the brain. Springer, New York, NY
42. Bhatia SN, Ingber DE (2014) Microfluidic organs-on-chips. Nat Biotechnol 32(8):760–772
43. Nag S (2003) Morphology and molecular properties of cellular components of normal cerebral vessels. Methods Mol Med 89:3–36
44. Bhalerao A, Sivandzade F, Archie SR, Chowdhury EA, Noorani B, Cucullo LJF et al (2020) In vitro modeling of the neurovascular unit: advances in the field. Fluids Barriers CNS 17(1): 1–20
45. Cucullo L, Couraud PO, Weksler B, Romero IA, Hossain M, Rapp E et al (2008) Immortalized human brain endothelial cells and flow-based vascular modeling: a marriage of convenience for rational neurovascular studies. J Cereb Blood Flow Metab 28(2):312–328
46. Booth R, Kim H (2012) Characterization of a microfluidic in vitro model of the blood-brain barrier (μBBB). Lab Chip 12(10):1784–1792
47. Achyuta AK, Conway AJ, Crouse RB, Bannister EC, Lee RN, Katnik CP et al (2013) A modular approach to create a neurovascular unit-on-a-chip. Lab Chip 13(4):542–553
48. Griep LM, Wolbers F, de Wagenaar B, ter Braak PM, Weksler BB, Romero IA et al (2013) BBB on chip: microfluidic platform to mechanically and biochemically modulate blood-brain barrier function. Biomed Microdevices 15(1):145–150

49. Brown JA, Pensabene V, Markov DA, Allwardt V, Neely MD, Shi M et al (2015) Recreating blood-brain barrier physiology and structure on chip: a novel neurovascular microfluidic bioreactor. Biomicrofluidics 9(5):054124
50. Sellgren KL, Hawkins BT, Grego S (2015) An optically transparent membrane supports shear stress studies in a three-dimensional microfluidic neurovascular unit model. Biomicrofluidics 9(6):061102
51. Maoz BM, Herland A, FitzGerald EA, Grevesse T, Vidoudez C, Pacheco AR et al (2018) A linked organ-on-chip model of the human neurovascular unit reveals the metabolic coupling of endothelial and neuronal cells. Nat Biotechnol 36(9):865–874
52. Oddo A, Peng B, Tong Z, Wei Y, Tong WY, Thissen H et al (2019) Advances in microfluidic blood-brain barrier (BBB) models. Trends Biotechnol 37(12):1295–1314
53. Prabhakarpandian B, Shen MC, Nichols JB, Mills IR, Sidoryk-Wegrzynowicz M, Aschner M et al (2013) SyM-BBB: a microfluidic blood brain barrier model. Lab Chip 13(6):1093–1101
54. Deosarkar SP, Prabhakarpandian B, Wang B, Sheffield JB, Krynska B, Kiani MF (2015) A novel dynamic neonatal blood-brain barrier on a chip. PLoS One 10(11):e0142725
55. Adriani G, Ma D, Pavesi A, Kamm RD, Goh EL (2017) A 3D neurovascular microfluidic model consisting of neurons, astrocytes and cerebral endothelial cells as a blood-brain barrier. Lab Chip 17(3):448–459
56. Xu H, Li Z, Yu Y, Sizdahkhani S, Ho WS, Yin F et al (2016) A dynamic in vivo-like organotypic blood-brain barrier model to probe metastatic brain tumors. Sci Rep 6:36670
57. van der Helm MW, Odijk M, Frimat JP, van der Meer AD, Eijkel JCT, van den Berg A et al (2016) Direct quantification of transendothelial electrical resistance in organs-on-chips. Biosens Bioelectron 85:924–929
58. Chrobak KM, Potter DR, Tien J (2006) Formation of perfused, functional microvascular tubes in vitro. Microvasc Res 71(3):185–196
59. Kim JA, Kim HN, Im S-K, Chung S, Kang JY, Choi N (2015) Collagen-based brain microvasculature model in vitro using three-dimensional printed template. Biomicrofluidics 9(2):024115
60. Alluri H, Shaji CA, Davis ML, Tharakan B (2018) A mouse controlled cortical impact model of traumatic brain injury for studying blood-brain barrier dysfunctions. Methods Mol Biol 1717:37–52
61. Sivandzade F, Prasad S, Bhalerao A, Cucullo L (2019) NRF2 and NF-B interplay in cerebrovascular and neurodegenerative disorders: molecular mechanisms and possible therapeutic approaches. Redox Biol 21:101059
62. Zhang BA (2016) Microfluidic approach to tissue vascularization. University of Toronto, Canada
63. Wiranowska M, Wilson TC, Beneze KS, Prockop LD (1988) A mouse model for the study of blood-brain barrier permeability. J Neurosci Methods 26(2):105–109
64. Rapoport SI, Bachman DS, Thompson HK (1972) Chronic effects of osmotic opening of the blood-brain barrier in the monkey. Science 176(4040):1243–1244
65. Sohet F, Daneman R (2013) Genetic mouse models to study blood–brain barrier development and function. Fluids Barriers CNS 10(1):1–18
66. Neumaier F, Zlatopolskiy BD, Neumaier B (2021) Drug penetration into the central nervous system: pharmacokinetic concepts and in vitro model systems. Pharmaceutics 13(10):1542
67. Hajal C, Campisi M, Mattu C, Chiono V, Kamm RD (2018) In vitro models of molecular and nano-particle transport across the blood-brain barrier. Biomicrofluidics 12(4):042213
68. D'Costa K, Kosic M, Lam A, Moradipour A, Zhao Y, Radisic M (2020) Biomaterials and culture systems for development of organoid and organ-on-a-chip models. Ann Biomed Eng 48(7):2002–2027
69. Fabre KM, Delsing L, Hicks R, Colclough N, Crowther DC, Ewart L (2019) Utilizing microphysiological systems and induced pluripotent stem cells for disease modeling: a case study for blood brain barrier research in a pharmaceutical setting. Adv Drug Deliv Rev 140: 129–135

70. Zheng Z, Chopp M, Chen J (2020) Multifaceted roles of pericytes in central nervous system homeostasis and disease. J Cereb Blood Flow Metab 40(7):1381–1401
71. Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, Hickman JJ (2015) TEER measurement techniques for in vitro barrier model systems. J Lab Autom 20(2):107–126
72. Crone C, Christensen O (1981) Electrical resistance of a capillary endothelium. J Gen Physiol 77(4):349–371
73. Avemary J, Salvamoser JD, Peraud A, Rémi J, Noachtar S, Fricker G et al (2013) Dynamic regulation of P-glycoprotein in human brain capillaries. Mol Pharm 10(9):3333–3341
74. Lochhead JJ, Yang J, Ronaldson PT, Davis TP (2020) Structure, function, and regulation of the blood-brain barrier tight junction in central nervous system disorders. Front Physiol 11:914
75. Lacombe O, Videau O, Chevillon D, Guyot A-C, Contreras C, Blondel S et al (2011) In vitro primary human and animal cell-based blood− brain barrier models as a screening tool in drug discovery. Mol Pharm 8(3):651–663
76. Gaston JD, Bischel LL, Fitzgerald LA, Cusick KD, Ringeisen BR, Pirlo RK (2017) Gene expression changes in long-term in vitro human blood-brain barrier models and their dependence on a transwell scaffold material. J Healthc Eng. https://doi.org/10.1155/2017/5740975
77. Cecchelli R, Berezowski V, Lundquist S, Culot M, Renftel M, Dehouck M-P et al (2007) Modelling of the blood–brain barrier in drug discovery and development. Nat Rev Drug Discov 6(8):650–661
78. Teixeira MI, Amaral MH, Costa PC, Lopes CM, Lamprou DA (2020) Recent developments in microfluidic technologies for central nervous system targeted studies. Pharmaceutics 12(6):542
79. Offeddu GS, Possenti L, Loessberg-Zahl JT, Zunino P, Roberts J, Han X et al (2019) Application of transmural flow across in vitro microvasculature enables direct sampling of interstitial therapeutic molecule distribution. Small 15(46):1902393
80. Offeddu GS, Haase K, Gillrie MR, Li R, Morozova O, Hickman D et al (2019) An on-chip model of protein paracellular and transcellular permeability in the microcirculation. Biomaterials 212:115–125
81. Bertassoni LE, Cecconi M, Manoharan V, Nikkhah M, Hjortnaes J, Cristino AL et al (2014) Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. Lab Chip 14(13):2202–2211
82. Eigenmann D (2015) Establishment and validation of an immortalized in vitro human blood-brain barrier (BBB) model for drug permeability studies, and application to natural product derived leads. University_of_Basel
83. Watanabe T, Dohgu S, Takata F, Nishioku T, Nakashima A, Futagami K et al (2013) Paracellular barrier and tight junction protein expression in the immortalized brain endothelial cell lines bEND. 3, bEND. 5 and mouse brain endothelial cell 4. Bio Pharm Bull 36(3):492–495
84. Brown RC, Morris AP, O'Neil RG (2007) Tight junction protein expression and barrier properties of immortalized mouse brain microvessel endothelial cells. Brain Res 1130:17–30
85. Eigenmann DE, Xue G, Kim KS, Moses AV, Hamburger M, Oufir MJF et al (2013) Comparative study of four immortalized human brain capillary endothelial cell lines, hCMEC/D3, hBMEC, TY10, and BB19, and optimization of culture conditions, for an in vitro blood–brain barrier model for drug permeability studies. Fluids Barriers CNS 10(1):1–17
86. Wevers NR, Kasi DG, Gray T, Wilschut KJ, Smith B, Van Vught R et al (2018) A perfused human blood–brain barrier on-a-chip for high-throughput assessment of barrier function and antibody transport. Fluids Barriers CNS 15(1):1–12
87. Vatsa P, Negi R, Ansari U, Khanna V, Pant AB (2021) Insights of extracellular vesicles of mesenchymal stem cells: a prospective cell-free regenerative medicine for neurodegenerative disorders. Mol Neurobiol:1–16. https://doi.org/10.1007/s12035-021-02603-7
88. Jahan S, Kumar D, Kumar A, Rajpurohit CS, Singh S, Srivastava A et al (2017) Neurotrophic factor mediated neuronal differentiation of human cord blood mesenchymal stem cells and

their applicability to assess the developmental neurotoxicity. Biochem Biophys Res Commun 482(4):961–967
89. Srivastava A, Vatsa P, Singh S, Kumar D, Pant AB (2018) Apoptotic response of human mesenchymal stem cells against dose dependent exposure to organophosphate pesticide-monocrotophos. J Ecophysiol Occup Health 17(3-4):106–113
90. Singh S, Srivastava A, Kumar V, Pandey A, Kumar D, Rajpurohit C et al (2016) Stem cells in neurotoxicology/developmental neurotoxicology: current scenario and future prospects. Mol Neurobiol 53(10):6938–6949
91. Rajpurohit CS, Kumar V, Cheffer A, Oliveira D, Ulrich H, Okamoto OK et al (2020) Mechanistic insights of astrocyte-mediated hyperactive autophagy and loss of motor neuron function in SOD1(L39R) linked amyotrophic lateral sclerosis. Mol Neurobiol 57(10): 4117–4133
92. Lippmann ES, Azarin SM, Kay JE, Nessler RA, Wilson HK, Al-Ahmad A et al (2012) Derivation of blood-brain barrier endothelial cells from human pluripotent stem cells. Nat Biotechnol 30(8):783–791
93. Orlova VV, Van Den Hil FE, Petrus-Reurer S, Drabsch Y, Ten Dijke P, Mummery CL (2014) Generation, expansion and functional analysis of endothelial cells and pericytes derived from human pluripotent stem cells. Nat Protoc 9(6):1514–1531
94. Hong YJ, Do JT (2019) Neural lineage differentiation from pluripotent stem cells to mimic human brain tissues. Front Bioeng Biotechnol 7:400
95. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K et al (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861–872
96. Lippmann ES, Al-Ahmad A, Palecek SP, Shusta EV (2013) Modeling the blood–brain barrier using stem cell sources. Fluids Barriers CNS 10(1):1–14
97. Qian T, Hernday SE, Bao X, Olson WR, Panzer SE, Shusta EV et al (2019) Directed differentiation of human pluripotent stem cells to podocytes under defined conditions. Sci Rep 9(1):1–12
98. Workman MJ, Svendsen CN (2020) Recent advances in human iPSC-derived models of the blood–brain barrier. Fluids Barriers CNS 17(1):1–10
99. Park T, Mustafaoglu N, Herland A, Hasselkus R, Mannix R, FitzGerald E et al (2019) Hypoxia-enhanced blood-brain barrier chip recapitulates human barrier function and shuttling of drugs and antibodies. Nat Commun 10(1):2621
100. Chen X, Liu C, Muok L, Zeng C, Li Y (2021) Dynamic 3D on-chip BBB model design, development, and applications in neurological diseases. Cells 10(11):3183
101. Edmondson R, Broglie JJ, Adcock AF, Yang L (2014) Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol 12(4):207–218
102. Perrot CY, Herrera JL, Fournier-Goss AE, Komatsu M (2020) Prostaglandin E2 breaks down pericyte-endothelial cell interaction via EP1 and EP4-dependent downregulation of pericyte N-cadherin, connexin-43, and R-Ras. Sci Rep 10(1):11186
103. Workman MJ, Svendsen CN (2020) Recent advances in human iPSC-derived models of the blood-brain barrier. Fluids Barriers CNS 17(1):30
104. Bagchi S, Chhibber T, Lahooti B, Verma A, Borse V, Jayant RD (2019) In-vitro blood-brain barrier models for drug screening and permeation studies: an overview. Drug Des Devel Ther 13:3591–3605
105. Wang X, Xu B, Xiang M, Yang X, Liu Y, Liu X et al (2020) Advances on fluid shear stress regulating blood-brain barrier. Microvasc Res 128:103930
106. Zhang B, Korolj A, Lai BFL, Radisic M (2018) Advances in organ-on-a-chip engineering. Nat Rev Mater 3(8):257–278
107. Lee S, Chung M, Lee SR, Jeon NL (2020) 3D brain angiogenesis model to reconstitute functional human blood–brain barrier in vitro. Biotechnol Bioeng 117(3):748–762

108. Brown TD, Nowak M, Bayles AV, Prabhakarpandian B, Karande P, Lahann J et al (2019) A microfluidic model of human brain (μHuB) for assessment of blood brain barrier. Bioeng Transl Med 4(2):e10126
109. Vatine GD, Barrile R, Workman MJ, Sances S, Barriga BK, Rahnama M et al (2019) Human iPSC-derived blood-brain barrier chips enable disease modeling and personalized medicine applications. Cell Stem Cell 24(6):995–1005.e6
110. Herland A, Maoz BM, FitzGerald EA, Grevesse T, Vidoudez C, Sheehy SP et al (2020) Proteomic and metabolomic characterization of human neurovascular unit cells in response to methamphetamine. Adv Biosyst 4(9):1900230
111. Vernetti L, Gough A, Baetz N, Blutt S, Broughman JR, Brown JA et al (2017) Corrigendum: Functional coupling of human microphysiology systems: Intestine, liver, kidney proximal tubule, blood-brain barrier and skeletal muscle. Sci Rep 7:44517
112. Novak R, Ingram M, Marquez S, Das D, Delahanty A, Herland A et al (2020) Robotic fluidic coupling and interrogation of multiple vascularized organ chips. Nat Biomed Eng 4(4): 407–420
113. Wu Q, Liu J, Wang X, Feng L, Wu J, Zhu X et al (2020) Organ-on-a-chip: Recent breakthroughs and future prospects. Biomed Eng Online 19(1):1–19
114. Probst C, Schneider S, Loskill P (2018) High-throughput organ-on-a-chip systems: Current status and remaining challenges. Curr Opin Biomed Eng 6:33–41
115. Ahn SI, Sei YJ, Park H-J, Kim J, Ryu Y, Choi JJ et al (2020) Microengineered human blood–brain barrier platform for understanding nanoparticle transport mechanisms. Nat Commun 11(1):1–12
116. Katt ME, Linville RM, Mayo LN, Xu ZS, Searson PC (2018) Functional brain-specific microvessels from iPSC-derived human brain microvascular endothelial cells: the role of matrix composition on monolayer formation. Fluids Barriers CNS 15(1):1–12
117. Marino A, Tricinci O, Battaglini M, Filippeschi C, Mattoli V, Sinibaldi E et al (2018) A 3D real-scale, biomimetic, and biohybrid model of the blood-brain barrier fabricated through two-photon lithography. Small 14(6):1702959
118. Rauti R, Renous N, Maoz BM (2020) Mimicking the brain extracellular matrix in Vitro: a review of current methodologies and challenges. Isr J Chem 60(12):1141–1151
119. Herland A, van der Meer AD, FitzGerald EA, Park T-E, Sleeboom JJ, Ingber DE (2016) Distinct contributions of astrocytes and pericytes to neuroinflammation identified in a 3D human blood-brain barrier on a chip. PLoS One 11(3):e0150360
120. Chen MB, Whisler JA, Fröse J, Yu C, Shin Y, Kamm RD (2017) On-chip human microvasculature assay for visualization and quantification of tumor cell extravasation dynamics. Nat Protoc 12(5):865–880
121. Peak CW, Cross L, Singh A, Gaharwar AK (2016) Microscale technologies for engineering complex tissue structures. Microscale technologies for cell engineering. Springer, New York, pp 3–25
122. Bang S, Lee S-R, Ko J, Son K, Tahk D, Ahn J et al (2017) A low permeability microfluidic blood-brain barrier platform with direct contact between perfusable vascular network and astrocytes. Sci Rep 7(1):1–10
123. Qian X, Song H, Ming GL (2019) Brain organoids: advances, applications and challenges. Development 146(8):dev166074
124. Raja WK, Mungenast AE, Lin Y-T, Ko T, Abdurrob F, Seo J et al (2016) Self-organizing 3D human neural tissue derived from induced pluripotent stem cells recapitulate Alzheimer's disease phenotypes. PLoS One 11(9):e0161969
125. Pham MT, Pollock KM, Rose MD, Cary WA, Stewart HR, Zhou P et al (2018) Generation of human vascularized brain organoids. Neuroreport 29(7):588
126. Cakir B, Xiang Y, Tanaka Y, Kural MH, Parent M, Kang Y-J et al (2019) Engineering of human brain organoids with a functional vascular-like system. Nat Methods 16(11): 1169–1175

127. Yi H-G, Jeong YH, Kim Y, Choi Y-J, Moon HE, Park SH et al (2019) A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. Nat Biomed Eng 3(7):509–519
128. Grifno GN, Farrell AM, Linville RM, Arevalo D, Kim JH, Gu L et al (2019) Tissue-engineered blood-brain barrier models via directed differentiation of human induced pluripotent stem cells. Sci Rep 9(1):1–13
129. Wilhelm I, Krizbai IA (2014) In vitro models of the blood–brain barrier for the study of drug delivery to the brain. Mol Pharm 11(7):1949–1963
130. Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, Kamiie J et al (2011) Quantitative targeted absolute proteomics of human blood–brain barrier transporters and receptors. J Neurochem 117(2):333–345
131. Uchida Y, Tachikawa M, Obuchi W, Hoshi Y, Tomioka Y, Ohtsuki S et al (2013) A study protocol for quantitative targeted absolute proteomics (QTAP) by LC-MS/MS: application for inter-strain differences in protein expression levels of transporters, receptors, claudin-5, and marker proteins at the blood–brain barrier in ddY, FVB, and C57BL/6J mice. Fluids Barriers CNS 10(1):1–22
132. Weksler B, Romero IA, Couraud P-O (2013) The hCMEC/D3 cell line as a model of the human blood brain barrier. Fluids Barriers CNS 10(1):1–10
133. Bhalerao A, Sivandzade F, Archie SR, Chowdhury EA, Noorani B, Cucullo L (2020) In vitro modeling of the neurovascular unit: advances in the field. Fluids Barriers CNS 17(1):22
134. Ponio JB-D, El-Ayoubi F, Glacial F, Ganeshamoorthy K, Driancourt C, Godet M et al (2014) Instruction of circulating endothelial progenitors in vitro towards specialized blood-brain barrier and arterial phenotypes. PLoS One 9(1):e84179
135. Cecchelli R, Aday S, Sevin E, Almeida C, Culot M, Dehouck L et al (2014) A stable and reproducible human blood-brain barrier model derived from hematopoietic stem cells. PLoS One 9(6):e99733
136. Bergmann S, Lawler SE, Qu Y, Fadzen CM, Wolfe JM, Regan MS et al (2018) Blood–brain-barrier organoids for investigating the permeability of CNS therapeutics. Nat Protoc 13(12): 2827–2843
137. Palmiotti CA, Prasad S, Naik P, Abul KM, Sajja RK, Achyuta AH et al (2014) In vitro cerebrovascular modeling in the 21st century: current and prospective technologies. Pharm Res 31(12):3229–3250
138. Cucullo L, McAllister MS, Kight K, Krizanac-Bengez L, Marroni M, Mayberg MR et al (2002) A new dynamic in vitro model for the multidimensional study of astrocyte–endothelial cell interactions at the blood–brain barrier. Brain Res 951(2):243–254
139. Yeon JH, Na D, Choi K, Ryu SW, Choi C, Park JK (2012) Reliable permeability assay system in a microfluidic device mimicking cerebral vasculatures. Biomed Microdevices 14(6): 1141–1148
140. Tourovskaia A, Fauver M, Kramer G, Simonson S, Neumann T (2014) Tissue-engineered microenvironment systems for modeling human vasculature. Exp Biol Med (Maywood) 239(9):1264–1271
141. Bonakdar M, Wasson EM, Lee YW, Davalos RV (2016) Electroporation of brain endothelial cells on chip toward permeabilizing the blood-brain barrier. Biophys J 110(2):503–513
142. Shao X, Gao D, Chen Y, Jin F, Hu G, Jiang Y et al (2016) Development of a blood-brain barrier model in a membrane-based microchip for characterization of drug permeability and cytotoxicity for drug screening. Anal Chim Acta 934:186–193
143. Partyka PP, Godsey GA, Galie JR, Kosciuk MC, Acharya NK, Nagele RG et al (2017) Mechanical stress regulates transport in a compliant 3D model of the blood-brain barrier. Biomaterials 115:30–39
144. Wang YI, Abaci HE, Shuler ML (2017) Microfluidic blood-brain barrier model provides in vivo-like barrier properties for drug permeability screening. Biotechnol Bioeng 114(1): 184–194

145. Tang Y, Soroush F, Sun S, Liverani E, Langston JC, Yang Q et al (2018) Protein kinase C-delta inhibition protects blood-brain barrier from sepsis-induced vascular damage. J Neuroinflammation 15(1):309
146. Wevers NR, Kasi DG, Gray T, Wilschut KJ, Smith B, van Vught R et al (2018) A perfused human blood-brain barrier on-a-chip for high-throughput assessment of barrier function and antibody transport. Fluids Barriers CNS 15(1):23
147. Jeong S, Kim S, Buonocore J, Park J, Welsh CJ, Li J et al (2018) A three-dimensional arrayed microfluidic blood-brain barrier model with integrated electrical sensor array. IEEE Trans Biomed Eng 65(2):431–439
148. Koo Y, Hawkins BT, Yun Y (2018) Three-dimensional (3D) tetra-culture brain on chip platform for organophosphate toxicity screening. Sci Rep 8(1):2841
149. Brown TD, Nowak M, Bayles AV, Prabhakarpandian B, Karande P, Lahann J et al (2019) A microfluidic model of human brain (muHuB) for assessment of blood brain barrier. Bioeng Transl Med 4(2):e10126
150. Liu W, Song J, Du X, Zhou Y, Li Y, Li R et al (2019) AKR1B10 (Aldo-keto reductase family 1 B10) promotes brain metastasis of lung cancer cells in a multi-organ microfluidic chip model. Acta Biomater 91:195–208
151. Buzhdygan TP, DeOre BJ, Baldwin-Leclair A, Bullock TA, McGary HM, Khan JA et al (2020) The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in-vitro models of the human blood-brain barrier. Neurobiol Dis 146:105131
152. Salman MM, Marsh G, Kusters I, Delince M, Di Caprio G, Upadhyayula S et al (2020) Design and validation of a human brain endothelial microvessel-on-a-chip open microfluidic model enabling advanced optical imaging. Front Bioeng Biotechnol 8:573775
153. Ahn SI, Sei YJ, Park HJ, Kim J, Ryu Y, Choi JJ et al (2020) Microengineered human blood-brain barrier platform for understanding nanoparticle transport mechanisms. Nat Commun 11(1):175
154. Abbott NJ (2004) Prediction of blood–brain barrier permeation in drug discovery from in vivo, in vitro and in silico models. Drug Discov Today Technol 1(4):407–416
155. Wolff A, Antfolk M, Brodin B, Tenje MJ (2015) In vitro blood–brain barrier models—an overview of established models and new microfluidic approaches. J Pharm Sci 104(9): 2727–2746
156. Huh D, Torisawa Y-S, Hamilton GA, Kim HJ, Ingber DE (2012) Microengineered physiological biomimicry: organs-on-chips. Lab Chip 12(12):2156–2164
157. Pamies D, Hartung T, Hogberg HT (2014) Biological and medical applications of a brain-on-a-chip. Exp Biol Med (Maywood) 239(9):1096–1107
158. Perrin S (2014) Preclinical research: Make mouse studies work. Nature 507(7493):423
159. van der Helm MW, van der Meer AD, Eijkel JC, van den Berg A, Segerink LI (2016) Microfluidic organ-on-chip technology for blood-brain barrier research. Tissue Barriers 4(1): e1142493
160. Van Der Meer AD, Van Den Berg A (2012) Organs-on-chips: breaking the in vitro impasse. Integr Biol 4(5):461–470
161. Abbott NJ, Dolman DE, Yusof SR, Reichel A (2014) In vitro models of CNS barriers. New York, Springer, pp 163–197
162. Hatherell K, Couraud P-O, Romero IA, Weksler B, Pilkington GJ (2011) Development of a three-dimensional, all-human in vitro model of the blood–brain barrier using mono-, co-, and tri-cultivation Transwell models. J Neurosci Methods 199(2):223–229
163. Logun M, Zhao W, Mao L, Karumbaiah L (2018) Microfluidics in malignant glioma research and precision medicine. Adv Biosyst 2(5):1700221
164. Chonan Y, Taki S, Sampetrean O, Saya H, Sudo R (2017) Endothelium-induced three-dimensional invasion of heterogeneous glioma initiating cells in a microfluidic coculture platform. Integr Biol (Camb) 9(9):762–773
165. Ayuso JM, Monge R, Martinez-Gonzalez A, Virumbrales-Munoz M, Llamazares GA, Berganzo J et al (2017) Glioblastoma on a microfluidic chip: Generating pseudopalisades

and enhancing aggressiveness through blood vessel obstruction events. Neuro Oncol 19(4): 503–513
166. Fan Y, Nguyen DT, Akay Y, Xu F, Akay M (2016) Engineering a brain cancer chip for high-throughput drug screening. Sci Rep 6:25062
167. Walker B Jr (2000) Neurotoxicity in human beings. J Lab Clin Med 136(3):168–180
168. Culot M, Lundquist S, Vanuxeem D, Nion S, Landry C, Delplace Y et al (2008) An in vitro blood-brain barrier model for high throughput (HTS) toxicological screening. Toxicol In Vitro 22(3):799–811
169. Wang H (2018) Modeling neurological diseases with human brain organoids. Front Synaptic Neurosci 10:15
170. Park J, Wetzel I, Marriott I, Dreau D, D'Avanzo C, Kim DY et al (2018) A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. Nat Neurosci 21(7):941–951
171. Wu X, Newbold MA, Gao Z, Haynes CL (2017) A versatile microfluidic platform for the study of cellular interactions between endothelial cells and neutrophils. Biochim Biophys Acta Gen Subj 1861(5 Pt A):1122–1130
172. Stowe AM, Adair-Kirk TL, Gonzales ER, Perez RS, Shah AR, Park TS et al (2009) Neutrophil elastase and neurovascular injury following focal stroke and reperfusion. Neurobiol Dis 35(1): 82–90
173. Amor S, Puentes F, Baker D, van der Valk P (2010) Inflammation in neurodegenerative diseases. Immunology 129(2):154–169
174. Cho H, Seo JH, Wong KH, Terasaki Y, Park J, Bong K et al (2015) Three-dimensional blood-brain barrier model for in vitro studies of neurovascular pathology. Sci Rep 5:15222
175. Brown JA, Codreanu SG, Shi M, Sherrod SD, Markov DA, Neely MD et al (2016) Metabolic consequences of inflammatory disruption of the blood-brain barrier in an organ-on-chip model of the human neurovascular unit. J Neuroinflamm 13(1):306
176. Chen Z, Ruan J, Li D, Wang M, Han Z, Qiu W et al (2020) The role of intestinal bacteria and gut-brain axis in hepatic encephalopathy. Front Cell Infect Microbiol 10:595759
177. Natarajan R, Northrop N, Yamamoto B (2017) Fluorescein isothiocyanate (FITC)-dextran extravasation as a measure of blood-brain barrier permeability. Curr Protoc Neurosci 79: 9.58.1–9.58.15
178. Terrell-Hall TB, Ammer AG, Griffith JI, Lockman PR (2017) Permeability across a novel microfluidic blood-tumor barrier model. Fluids Barriers CNS 14(1):3
179. Qiu Y, Ahn B, Sakurai Y, Hansen CE, Tran R, Mimche PN et al (2018) Microvasculature-on-a-chip for the long-term study of endothelial barrier dysfunction and microvascular obstruction in disease. Nat Biomed Eng 2:453–463
180. Song JW, Munn LL (2011) Fluid forces control endothelial sprouting. Proc Natl Acad Sci U S A 108(37):15342–15347
181. Bang S, Lee SR, Ko J, Son K, Tahk D, Ahn J et al (2017) A low permeability microfluidic blood-brain barrier platform with direct contact between perfusable vascular network and astrocytes. Sci Rep 7(1):8083
182. Butovsky O, Weiner HL (2018) Microglial signatures and their role in health and disease. Nat Rev Neurosci 19(10):622–635
183. Shawahna R, Decleves X, Scherrmann JM (2013) Hurdles with using in vitro models to predict human blood-brain barrier drug permeability: a special focus on transporters and metabolizing enzymes. Curr Drug Metab 14(1):120–136
184. Weksler B, Romero IA, Couraud PO (2013) The hCMEC/D3 cell line as a model of the human blood brain barrier. Fluids Barriers CNS 10(1):16
185. Eigenmann DE, Xue G, Kim KS, Moses AV, Hamburger M, Oufir M (2013) Comparative study of four immortalized human brain capillary endothelial cell lines, hCMEC/D3, hBMEC, TY10, and BB19, and optimization of culture conditions, for an in vitro blood-brain barrier model for drug permeability studies. Fluids Barriers CNS 10(1):33

186. Lin S, Lin Y, Nery JR, Urich MA, Breschi A, Davis CA et al (2014) Comparison of the transcriptional landscapes between human and mouse tissues. Proc Natl Acad Sci U S A 111(48):17224–17229
187. Shi Y, Inoue H, Wu JC, Yamanaka S (2017) Induced pluripotent stem cell technology: a decade of progress. Nat Rev Drug Discov 16(2):115–130
188. Dauth S, Maoz BM, Sheehy SP, Hemphill MA, Murty T, Macedonia MK et al (2017) Neurons derived from different brain regions are inherently different in vitro: a novel multiregional brain-on-a-chip. J Neurophysiol 117(3):1320–1341
189. Sances S, Ho R, Vatine G, West D, Laperle A, Meyer A et al (2018) Human iPSC-derived endothelial cells and microengineered organ-chip enhance neuronal development. Stem Cell Rep 10(4):1222–1236
190. Palus M, Vancova M, Sirmarova J, Elsterova J, Perner J, Ruzek D (2017) Tick-borne encephalitis virus infects human brain microvascular endothelial cells without compromising blood-brain barrier integrity. Virology 507:110–122
191. Papa MP, Meuren LM, Coelho SVA, Lucas CGO, Mustafa YM, Lemos Matassoli F et al (2017) Zika virus infects, activates, and crosses brain microvascular endothelial cells, without barrier disruption. Front Microbiol 8:2557
192. Allwardt V, Ainscough AJ, Viswanathan P, Sherrod SD, McLean JA, Haddrick M et al (2020) Translational roadmap for the organs-on-a-chip industry toward broad adoption. Bioengineering (Basel) 7(3):112
193. Serlin Y, Shelef I, Knyazer B, Friedman A (2015) Anatomy and physiology of the blood–brain barrier. Semin Cell Dev Biol. https://doi.org/10.1016/j.semcdb.2015.01.002
194. Odijk M, van der Meer AD, Levner D, Kim HJ, van der Helm MW, Segerink LI et al (2015) Measuring direct current trans-epithelial electrical resistance in organ-on-a-chip microsystems. Lab Chip 15(3):745–752
195. Thuenauer R, Rodriguez-Boulan E, Römer W (2014) Microfluidic approaches for epithelial cell layer culture and characterisation. Analyst 139(13):3206–3218
196. Benson K, Cramer S, Galla H-J (2013) Impedance-based cell monitoring: barrier properties and beyond. Fluids Barriers CNS 10(1):1–11
197. Zhang W, Zhu L, An C, Wang R, Yang L, Yu W et al (2020) The blood brain barrier in cerebral ischemic injury–Disruption and repair. Brain Hemorrhages 1(1):34–53
198. Ferrari E, Palma C, Vesentini S, Occhetta P, Rasponi M (2020) Integrating biosensors in organs-on-chip devices: a perspective on current strategies to monitor microphysiological systems. Biosensors 10(9):110
199. Syama S, Mohanan PV (2021) Microfluidic based human-on-a-chip: A revolutionary technology in scientific research. Trends Food Sci Technol 110:711–728
200. Low LA, Mummery C, Berridge BR, Austin CP, Tagle DA (2021) Organs-on-chips: into the next decade. Nat Rev Drug Discov 20(5):345–361
201. Vignon A, Salvador-Prince L, Lehmann S, Perrier V, Torrent J (2021) Deconstructing Alzheimer's disease: how to bridge the gap between experimental models and the human pathology? Int J Mol Sci 22(16):8769
202. Cameron T, Bennet T, Rowe EM, Anwer M, Wellington CL, Cheung KC (2021) Review of design considerations for brain-on-a-chip models. Micromachines (Basel) 12(4):441
203. van der Helm MW (2005) Electrical and microfluidic technologies for organs-on-chips. 19(1): 5–6

Multi-Organs-on-a-Chip in Disease Modelling

25

Tejaswini Appidi, Sushma Mudigunda, and Aravind Kumar Rengan

Abstract

Multi-organs-on-a-chip are microfluidic devices with multiple organs integrated on a single device, made up of cells of desired tissue/organ, enabling communication between organs, simulating the physiological environment. These chips are physiologically more relevant and show enhanced predictability of the efficacy and toxicity of drugs. These devices offer flexibility, affordability, reproducibility, and can be applied for personalized and precision medicine. Various physiological mechanisms that could not be understood using in vitro and in vivo models can be realized using the multi-organs-on-chip devices. This chapter discusses the application of multiple organs-on-a-chip for disease modelling. The microfluidic devices for cancer and cancer metastasis, infectious diseases are discussed in detail in this chapter. The efficacy of drugs and their off-toxicity is also discussed and compared to the conventional in vivo models.

Keywords

Microfluidics · Multi-organs · Disease modelling · Cancer · Metastasis · Infectious diseases

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25.1 Introduction

Multi-organs-on-a-chip is a great platform for disease modelling, redefining healthcare research for drug development, and testing their efficacy and toxicity. Major advances in drug development were limited by the non-availability of appropriate testing models and dependency on in vivo models which do not exactly mimic the human physiology. The development of novel drugs and their testing require high cost and microfluidic platforms have proven to be a powerful and flexible tool for simulating the required tissue/organ environment and perform experiments. Different cells/tissue models of various origins could be simulated and maintained with stable extracellular conditions. Multi-organs-on-a-chip has emerged as a successful model, facilitating interactions between multiple organs, aiding the testing of efficacy of novel drugs and formulations.

Cell-based models, which have been in use for several decades, have significant shortcomings: cell lines are cultured mostly in monolayer format which doesn't represent the dynamic 3D environment of the human body; cell lines fail to mimic cell and extracellular matrix interactions; cell lines undergo mutations; making it difficult to interpret or reproduce results [1]. In addition to these, various primary cells cannot be cultured for longer passages [2]. Also, the cultures are static, limiting the application of chemical/physical stimuli [3]. In vitro studies lack the vascular flow, extracellular microenvironment of tissues, organs, and doesn't mimic the multi-organ physiology, limiting its application for disease modelling and testing of drugs. The effect of the drug or disease cannot be understood just by monolayer culture of cells. It includes signalling molecules, complex responses, and pharmacokinetic behaviour which cannot be modelled using in vitro culture technology, making it difficult to study and understand the molecular mechanism, pathogenesis, and effects of drugs [4].

The animal models, especially mice models, have been very helpful and beneficial in understanding the efficacy of drugs and formulations. The molecular mechanisms, disease states involving tissue and organ level structural and functional changes, and interplay among multiple organs was effectively understood through animal models. These models have been employed for many years, yet they do not fully replicate the full spectrum of human phenotypes and are time-consuming and expensive. The animal models, including genetically engineered mice, can mimic the disease phenotypes, but the underlying cellular and physiological mechanisms are quite distinct as compared to humans, which is one of the reasons for the failure of various drugs and vaccines in human clinical trials, even after their successful efficacy in non-human primates. In addition to these, animal models lack experimental precision, and stricter ethical constraints restrict the development of various disease models and long term studies, requiring the need for more suitable and feasible preclinical models [4–7].

Cross-organ communication is essential to understand and decipher the efficacy and side effects of the drugs on various physiological functions. Various diseases such as sepsis, neurodegenerative diseases, infectious diseases, and cancer involve multiple organs and need accurate models for identifying biomarkers in body fluids

for diagnostic purposes. Tumour tissues release various biomarkers like circulating tumour cells, exosomes, etc., which play an important role in understanding the cancer and metastasis. In vivo models were the desired models to understand the interaction between various organs and effect of drugs or new formulations. In addition to the number of limitations of in vivo models as stated above, the animal models do not allow inter-organ cross talk analysis, determination of quantitative pharmacokinetics, and prediction of ADMET parameters (absorption/distribution/metabolism/excretion/toxicity) [3, 4, 8]. Hence, development of advanced and appropriate approaches incorporating multiple organs and allowing the analysis of all the parameters that decide the efficacy and toxicity of the drugs/new molecules is necessary for increased success rate of clinical trials.

Microfluidic organs-on-chip are dynamic microphysiological systems that can simulate vascular perfusion, tissue–tissue interfaces, organ-relevant functionalities, integrating circulating immune cells, and connective tissue cells which could serve as potential alternatives to various kinds of animal models. The most important advantage of microfluidic organ chips is the dynamic flow enabled by endothelium channels offering back and forth transfer of nutrients, wastes, and drugs, simulating an in vivo environment [4]. Multi-organ-on-chips are microfluidic devices that connect several tissue cultures on a chip to represent the function of different organs, allowing the observation of effects of a disease or drug on individual organs and the influence/response on other organs [9, 10]. The tissue–tissue and organ–organ interactions simulated using microfluidic devices offer physiologically relevant simulation with design control of fluid circuitry and chamber sizes [11]. Multi-organs-on-a-chip devices are capable of mimicking human metabolism, unlike animal studies, and allow a deeper analysis and interpretation of physiological processes, effects, and responses. The extrapolation of data for understanding the concentration of drugs/metabolites will also be more effective as compared to animal models. In addition to these, multi-organs-on-a-chip facilitates testing of various combinations of drugs in a parallel fashion, eliminating the requirement of large groups of animals for the same. These multi-organ chips can be redesigned as per the experimental requirements as we have full control over the design and fabrication of the devices. They are economical and can be used under both physiological and non-physiological conditions. The experimental results can be simulated and the predictions of these chips can be compared using mathematical models which help in filling the gaps between theoretical and experimental studies. The multi-organs-on-a-chip device facilitates affordable drug discovery by predicting the bioavailability, efficacy, and toxicity. These devices can be used for simulating various disease conditions by exploiting the design-and-flow characteristics and generate patient specific responses to drugs and therapeutics [10, 12].

Multi-organs-on-a-chip platform can be realized in two ways: by coupling of organ-on-chips or by integration of multiple organs onto a single chip [13]. Multi-organs-on-chip offer immense opportunities for developing complex disease models, investigating therapeutic efficacy, and understanding the adverse side effects, leading us towards more personalized medicine approaches. In this chapter, we discuss the multi-organs-on-a-chip for disease modelling. We introduce

multi-organ chip technology and its application for modelling of cancer, cancer metastasis, and infectious diseases. The efficacy of different drugs in these disease models is also discussed.

25.2 Cancer and Cancer Metastasis

Cancer is the one of the most common causes of morbidity and mortality globally. As per the International Agency for Research on Cancer (IARC) report, in the year 2020, 19.2 million new cases were reported and the total number of cancer deaths was 9.9 million [14, 15]. Diagnosis and treatment of cancer are the most important clinical problems, and various attempts have been made by researchers worldwide to understand the causes and identify the markers to facilitate early diagnosis and develop novel approaches for a safe treatment. The tumour micro-environment possesses various biochemical and biophysical parameters which cannot be mimicked by in vitro or in vivo models [16]. The main challenges in cancer research are developing models mimicking the tumour and its micro-environment similar to human physiology. It is these models that would enable us to study and understand the causes for its occurrence and various mechanisms resulting in its spread to different organs. Only with this knowledge we can design newer approaches to identify and treat tumours. Multi-organs-on-chip technology will be of great use in integrating various organs and mimicking the tumour environment, better than the in vitro or in vivo models. A few examples are discussed in this section (Fig. 25.1).

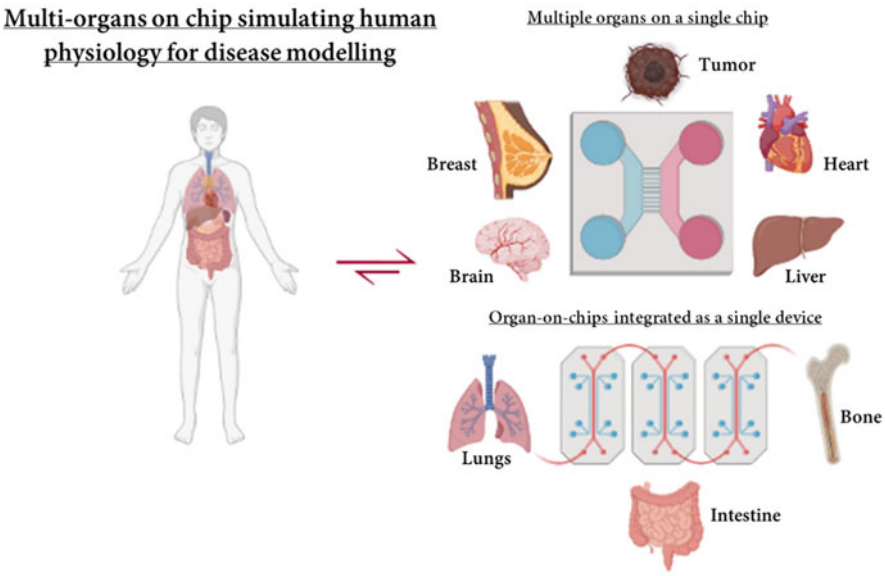

Fig. 25.1 Schematic showing the multi-organs-on-chip mimicking the human physiology. (Image prepared using biorender.com)

A microfluidic device mimicking the physiologically similar pharmacokinetic model was developed by Tatosian et al. to evaluate the response of drugs and molecules. The microfluidic device had cells representing liver (HepG2/C3A), bone marrow (MEG-01), uterine cancer (MES-SA), and multidrug-resistant (MDR) uterine cancer (MES-SA/DX-5). This microfluidic device, when treated with a combination of chemotherapeutics, showed selective inhibition of MDR cancer cells with acceptable levels of side effects. The microfluidic device can be used for studying the drug dosage levels for human trials and can be used for testing toxic and metabolic interactions [17]. Satoh et al. demonstrated a two organ system (liver and colorectal cancer) and a four organ system (liver, intestine, colorectal cancer, and connective tissue) for evaluating the efficacy of metabolism-dependent anticancer prodrugs (Capecitabine [CAP], tegafur [T], 5-fluorouracil [5-FU]). Liver has an important role in the bioactivation of many drugs, production of metabolites, and their clearance, and hence it is important to study the efficacy of the drugs in the presence of liver so as to mimic the physiological environment [18]. CAP was found to be successfully metabolized in the presence of HepaRG liver cells in the two-organ system, inhibiting the growth of cancer cells. The effect of three prodrugs, CAP, FT, and 5-FU, were evaluated, including hepatic metabolism, intestinal absorption, inhibition of growth of cancer cells, and connective tissue was observed in the four-organ system [19].

A microfluidic device was reported by Muñoz et al., involving co-culture of 3D breast cancer model and endothelium for evaluation of cross-talk and drug-delivery efficacy. The MDA-MB 231 cells in 3D collagen matrix served the purpose of breast cancer model, while HUVEC (human umbilical vein endothelial) cells served as endothelial barrier [20]. The microfluidic device successfully demonstrated all the key characteristics of tumour tissues like selective proliferation of cells with access to oxygen and nutrients, similar to in vivo models. In addition to this, the co-culture of cancer cells and endothelial cells resulted in a leaky vasculature, similar to tumour tissues in vivo. The microfluidic device was also evaluated for its drug delivery efficacy in the breast cancer model, depicting the potential of microfluidic devices for testing of precision medicine. A multi-organ system was developed and its versatility was demonstrated using two different examples, showing the efficacy and off-target toxicity of anti-cancer drugs. For the first application, the multi-organ-chip was cultured with primary hepatocytes and two types of cancer-derived human bone marrow cell lines. The anti-leukaemia drugs: diclofenac and imatinib showed a cytostatic effect, inhibiting cancer proliferation. Imatinib did not affect the viability of hepatocytes, while diclofenac showed reduced liver viability (by 30%). The microfluidic device's versatility was demonstrated by reconfiguring it and applying it for evaluating the combinatorial drug therapy on multidrug-resistant (MDR) vulva cancer line and non-multidrug-resistant breast cancer line in the presence of hepatocytes, and cardiomyocytes. Tamoxifen showed reduced viability of the breast cancer cells, but did not show any effect on the vulva cancer cells, when administered alone. Tamoxifen, when co-administered with verapamil, showed significant cytotoxic effect on both breast cancer and vulva cancer cells with

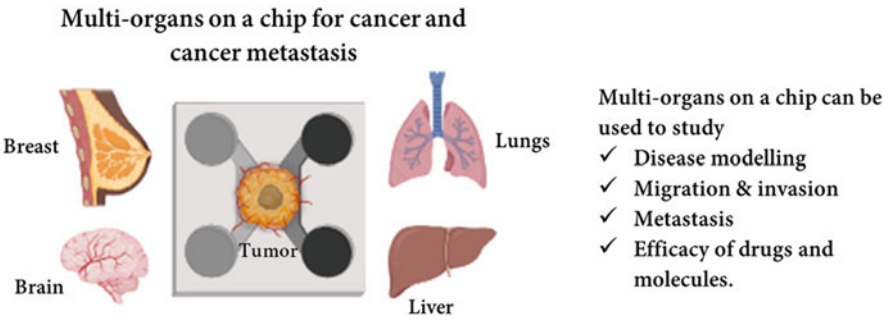

Fig. 25.2 Schematic showing the multi-organs-on-chip for cancer and cancer metastasis. (Image prepared using biorender.com)

off-target cardiac effects [21]. These microfluidic devices could be effectively applied as preclinical models for personalized medicine applications.

Cancer metastasis is caused by intravasation and colonization of circulating tumour cells in different organs [22]. Various processes, leading to metastasis, prediction of organs that would be affected, and design of new approaches for treatment of cancer metastasis, require integration of multiple organs, which could be realized using multiple organ-on-chips technology. Following are the few examples discussing the application of multi-organs-on-chip for understanding metastasis. A chemotherapeutic testing model using backward-compatible Tetris-Like (TILE) modular microfluidic platform was developed by Ong et al. and demonstrated the efficacy of liver-mediated bioactivation of cyclophosphamide against OSCC primary and metastatic tumour. A cancer model, with liver, endothelium, as well as primary and metastatic OSCC tumour modules, was designed. Primary and metastatic OSCC (patient-derived) 3D micro tumour modules were generated and connected to the 3D liver module. The cyclophosphamide resistance of the metastatic OSCC tumour was demonstrated to be overcome using the liver-mediated bioactivation [23]. This could not have been possible using the animal models and also as the model included human liver- and patient-derived primary and metastatic OSCC tumours, this device has the potential for point-of-care applications (Fig. 25.2).

Xu et al. developed a multi-organ microfluidic chip, including lungs and three different organs (brain, bone, and liver). Their study demonstrated the lung cell metastasis to brain, bone, and liver in the microfluidic chip and validated it with a nude mice model, proving the potential of the developed multi-organ-on-chip technology as an affordable and effective alternative to in vivo models [24]. The lungs and lung cancer were mimicked by growing bronchial epithelial, lung cancer, microvascular endothelial, mononuclear, and fibroblast cells resembling upstream "lung", while astrocytes, osteocytes, and hepatocytes were grown to mimic the brain, bone, and liver in distant chambers. The lung cancer cells showed epithelial to mesenchymal transition, indicating their metastatic behaviour. The astrocytes, osteocytes, and hepatocytes showed overexpression of CXCR4, RANKL, AFP

proteins, respectively, indicating metastasis to brain, bone, and liver, respectively. This system effectively demonstrated the growth of lung cancer, invasion, and metastasis.

A microfluidic model was developed by Kong et al., mimicking metastasis of circulating tumour cells (CTCs) to various organs. The primary cells from organs were cultured on a chip, representing the different organs; breast and salivary gland tumour cells were allowed to flow through these chambers to mimic the dynamic flow of CTCs. The CTCs metastasized to lungs, liver, and bone, in agreement with the experimental results from nude mice model. In addition to understanding the metastasis by CTCs to different organs, the same microfluidic chip was used to demonstrate efficacy of anti-metastatic agents, inhibiting the lung metastasis of breast cancer cells. Similar effects were observed in vivo, indicating the potential of this technology for establishing alternative metastasis models and understanding the effects of drugs [25].

Toh et al. designed and developed a microfluidic device for simulating cancer cell migration and invasion. This model facilitates the formation of three dimensional cellular aggregates, mimicking the cancer tumours before their migration and invasion. The basement membrane around the tumour was modelled using collagen, and real time migration and invasion of metastatic breast cancer cells from tumour were observed using this model. The breast cancer cells exhibited amoeboid-like motility with amorphous morphology and mesenchymal motility with elongated morphology. This device can be used as an alternative for understanding the cancer cell migration and evaluating the effect of drugs targeting the cell motility. This cell migration device has the potential to be used to study the loss of cell adhesion, ECM degradation and testing of anti-metastasis drugs [26]. Aleman et al. designed a multi-site metastatic microfluidic chip for assessing the metastatic preference of cancer cells. The device houses multiple 3D organoid sites, namely, colorectal cancer organoid residing in a single chamber, connected to liver, lung, and endothelial chambers downstream. The tumour cells grew in the cancer organoid, entered circulation, and were found to preferentially accumulate in the liver and lung organoids. This device facilitates tracking of the cells from the primary tumour organoid through circulation to potential sites of metastasis. This study concluded that despite the random shuffling of on-chip organoids' location, the cancer cells have a preference for metastasizing to lung and liver constructs [27]. Mao et al. designed two types of vascularized organs, built independently within a single system and verified its performance for studying organ-specific metastasis [28]. The device cultured with human hepatoma G2 cells (HepG2s), mesenchymal stem cells, and HUVEC cells showed formation of vasculature. Organ-specific metastasis was understood by perfusion of pancreatic cancer cells. The cells seeded into two halves of vascular system, formed functional vascularized organs, namely, liver and bone. Following the introduction and culture of pancreatic cancer cells, metastasized colonization was observed in liver and bone with significant number of pancreatic cells in liver as compared to bone. Thus, this human-on-leaf device could therefore successfully simulate a vascularized system which could be applied for understanding the metastasis in a vascularized environment. The multi-organs-on-a-

chip technology facilitates various advantages as compared to the conventional models. There is a greater scope of incorporation of further complexities in the devices to exactly mimic the human physiology, as required for various types of tumours and target organs. Though these devices have been greatly helpful in understanding the various mechanisms, enabling cross-organ communication with vascularized organs, design and fabrication of these devices are quite challenging and complex.

25.3 Infectious Diseases

Infectious diseases affect public health and economy worldwide. A variety of microbial pathogens (viruses, bacteria, fungi) invade the human body, causing serious illnesses and many infectious diseases. In recent times, the emergence of corona virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), called COVID-19 pandemic, has created a great loss economically, with an increase in the mortality rate [29]. Unlike other respiratory infectious diseases, COVID-19 has proven to be more communicable and fatal, with various symptoms, and damaging several organs. The emergence of human immunodeficiency virus (HIV) in the 1980s is still a major concern having worldwide mortality. Various epidemics and pandemics from many centuries like influenza virus H1N1, cholera, and bubonic plague in multiple outbreaks affected human lives. As the respiratory system in humans is more inclined to foreign pathogen interactions, the most pandemics are due to viruses like influenza, adeno virus, and corona virus. Among those, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome coronavirus (MERS) together account for high mortality rates. On the other hand, bacterial and parasitic diseases have a similar impact. Tuberculosis is another potentially serious infection caused by bacteria and is very contagious [30].

The disease is transmitted through droplets in air from the infected person, infecting the lungs. Moreover, the disease diagnosis at the early stages is difficult as the symptoms are mild for many months and the infection increases mortality in more immune compromised individuals. Malaria and hepatitis virus affect the hepatocytes in the liver and these infections still remain fatal globally. Hepatitis B, C virus infections causes more deaths across the globe with serious complications of liver cirrhosis. The condition is the same with malaria as stated by WHO, as the malaria cases have not been considerably changed over a period of time. An advance in the treatment requires understanding the human pathogen interactions and responses. The human gut is also infected with various bacteria, parasites, and viruses that lead to severe gastroenteritis and inflammations [31]. The human enteroviruses, like poliovirus, Coxsackie viruses, and echoviruses replicate in the gastrointestinal tract and mucous layer surfaces and Coxsackie virus B (CVB) disrupts the villi and integrity of the complete epithelium and enters the intestines.

Disease diagnosis of infections needs special attention and should be more robust, fast, and reliable to replicate the infections, helping in drug discovery and vaccine development. Various studies like tissue engineering and use of animal models for

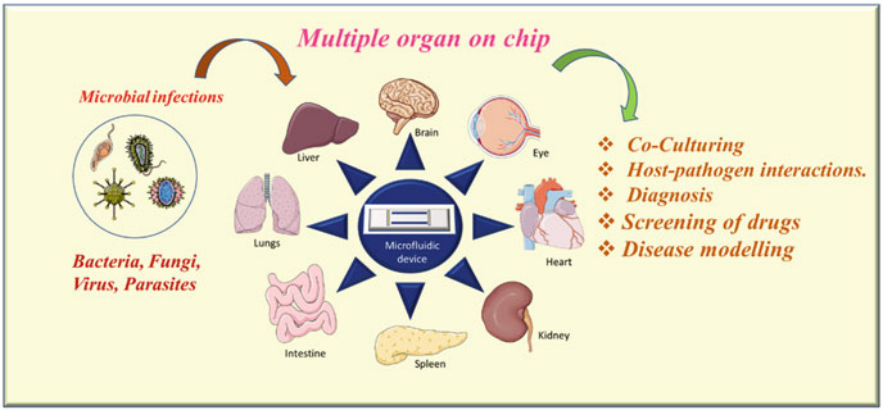

Fig. 25.3 Schematic representation of multiple organ-on-chip for infectious disease models

human cell culture to know the host pathogen interactions and drug testing in vitro is difficult, as 2D monolayers in cell cultures differ from the actual human processes in terms of epigenetics, genetic make-up, and various tissue and organ function [29]. The conventional methods for knowing the infectious diseases includes the morphological microscopic techniques, cell culturing, checking for antimicrobial agents, and use of animal models. Cell culturing in vitro is a very important method in understanding the host–pathogen interactions for many infectious diseases caused by parasites, hepatitis virus, and tuberculosis. But 2D cell culture may not provide the actual functioning of human tissues and organs and cannot replicate human responses and physiology [29]. The use of animal models for studying various organism responses to the infectious diseases, pathogenesis, and for drugs/vaccines efficacy are not useful because there is no evolutionary relativeness with humans.

An emerging technology that addresses many problems concerning replication of human physiology and use of low volume of bodily fluids and reagents along with the rapid detection of infectious disease pathogens is possible by microfluidic technologies. Paper microfluidic assays are the best example for single-step, user-friendly point-of-care solutions for relatively fast and equipment-free diagnosis of infectious disease detection [32]. Researchers take a step forward in view of the problems faced due to the emergence of pandemics over the years, and for developing new drugs and understanding the human responses to those infections, the microfluidic devices to replicate the in vivo functioning of different organs and understanding various epigenetics and gene profiles, a new technology called organ-on-a-chip has emerged. With the microfluidic organ chips, there is a scope for mimicking the actual in vivo environment with the controlled fluidic flow and maintaining the epigenetics and tissue–organ functions (Fig. 25.3).

Organ-on-a-chip or Organ chip has been used in many applications, especially for evaluating drugs, as they mimic many human organ models like lung, liver, heart, and intestines/gut. Recently, organ chips are used as the platforms for understanding host–pathogen interactions, host immune responses to various infectious diseases.

For example, a primary human hepatocytes chip has been used to know the immune response, similar to human liver, for hepatitis B infection. Hepatitis B is a very serious liver infection which is responsible for most of the liver infections that lead to liver cirrhosis and even liver cancers [31]. The first study mimicking the human liver on a chip was reported in 2015 for understanding the HBV (Hepatitis-B) replication. Primary rat hepatocytes on one side of the microporous membrane is used and bovine aortic endothelial cells were on the other side in a dual microchannel with continuous pumping of the medium. They successfully infected the cells with HBV genome and the cell viability and morphology was maintained for 26 days. The expression of HBV antigen and the DNA was observed after infection.

Gut-on-a-chip or intestines-on-a-chip helps in understanding the infections caused by various bacteria, parasites, and viral infections leading to inflammation or gastroenteritis. Human gastrointestinal (GI) tract is a complex system and in vitro modelling is more challenging. The organ chip helps in understanding enterovirus infections, release of new virions into epithelium in a controlled environment. A study showed how an enterovirus, Coxsackie virus B1 (CVB1) can effectively infect polarized intestinal epithelial cells when inoculated on either their apical and basolateral surfaces and observed production of cytokine after infection in the human gut-on-a-chip model [33]. Thus, this model provides a possible way for understanding a broad range of enteroviruses in a more realistic way that is very close to in vivo environment and mechanisms, thus helping more in drug development and for vaccines [31].

A wide range of clinical conditions and infections attack the human body through the respiratory route and lungs are the first and foremost affected organ. An effective way for early diagnosis and for drug development involves advanced technologies, and “Lung-on-a-chip” models provide a robust and reliable way to perform and understand in vivo functioning and processes [34]. Tuberculosis, a highly infectious disease, caused by small amount of droplets through air and even a very low concentration of bacterial cells in the droplets can lead to infection. It is difficult to know the infection in the initial stages and, in some cases, as a first line of defence, molecules called ‘surfactants’ are produced by the lungs and studying these molecules is even more difficult. In a study, to know the role of these surfactants as an immune response to tuberculosis, scientists used lung-on-a-chip technology. The chip was made of polymer compatible with the biological tissue and are made with a layer of cells for continuous circulation of air and blood flow. The results showed no bacterial infection when the surfactants were inoculated and sudden appearance of infection on their withdrawal. These finding give the idea of the protective role of surfactants and further open a new way to study the early stages of Mycobacterium tuberculosis infection, and help in controlling the disease [30].

In the recent pandemic of COVID-19, caused by SARS-CoV-2 virus, one of the main and primary organs which is mostly affected are lungs. Lung-on-a-chip reflects the actual human system for research. In a study, to know how native SARS-CoV-2 infects the alveolar barrier, a lung-on-a-chip with human pulmonary alveolar epithelial cells and microvascular epithelial cells is made and are infected with the native virus particles and found that the alveolar epithelium has been infected and

also replication of the virus occurred at the site. This study also showed that the virus could infect the epithelium but not the vascular endothelium. But the studies show that there has been lung vascular damage in COVID-19 patients, and the study states that the vascular damage was caused by the cytokines from the immune cells and not by the virus itself, and from further studies, the role of immune cells against the viral attack was understood. The work on human lung-on-a-chip helped in a better understanding of the COVID-19 infection [29]. Lung-on-a-chip has also been used for assessing the antiviral drugs against COVID-19. An FDA-approved drug, Remdesivir, was evaluated for its efficacy. The antiviral drug is effective for most of the RNA viruses, including SARS, MERS, etc. [29]. The drug is inoculated on to the epithelial channel on the chip and was able to inhibit the viral replication after 3 days of administration. This proves that lung chip models help in further screening of various antiviral drugs and vaccines against COVID-19.

Organ chip technology has been greatly beneficial in understanding the infectious disease states and the responses and damage at organ level. The study still faces many challenges in integrating other parameters that help in understanding more about the infectious diseases that are emerging and re-emerging from time to time. Further, the infectious diseases have systemic symptoms and interactions of multiple organs are essential for better understanding of human physiology and their responses. Multi organ-on-a-chip (multi-OoC) supports cross-organ interaction and allows modelling of diseases. The challenges involve maintaining the balance of multiple organs and other important functions, like immune responses, organ vascularization, hormonal stimulation, which cannot be acheived using in vitro modelling. Multi organ-on-a-chip devices can be used in future as patient-specific, to develop personalized models and with a knowledge of patients genetic and ethnic background, the drug interactions, side effects can be used for predicting the patient's responses to treatment [3].

Human stem cells organoids are another important in vitro model which retains most of the multicellular functions of the donor. These studies helped in the study of pathogenesis of various viruses like ZIKA, HBV, and even SARS-CoV-2. The organoid studies lack the immune cells, which is difficult for studying the immune responses against the particular pathogens. So, a combination of stem cells and human organ chips will improve the studies in vitro. The organ chips can be used for the monitoring of various environmental parameters and other biological processes. These can also be integrated with 3D printing, gene editing, and imaging. During recent times, a complex modelling system was developed by integrating multiple systems for understanding various infectious diseases and their effects on public health. Thus the organ chips and multi-organ chips can further be studied with more advances for better understanding of the infectious diseases and help in the control of epidemics and which is useful for human health.

25.4 Conclusions

Multi-organs-on-chip technology has a great potential and possibility in developing models mimicking physiology using human derived cells, which is a more appropriate and suitable model as compared to the existing and conventional in vitro and in vivo models. These models offer affordability and reproducibility for developing various disease models and testing the efficacy of drugs and new formulations. Microenvironments and cross-organ communication can be not only easily achieved, but the analysis and interpretation of various signalling molecules and processes is plausible with multi-organs-on-chip technology. In addition to all the characteristics and features provided by the existing models, these devices are also not limited by ethical constraints and hence disease models can be developed and cultured for longer durations to understand the systemic effects and changes in chronic diseases and to test the long-term effects of drugs and novel formulations. This chapter discussed the multi-organs-on-chip technology for the understanding cancer and its metastasis along with evaluation of different drugs and their effects on the diseased organ and other organs. This chapter also discussed the devices for modelling of infectious diseases, focusing on COVID 19. The disease modelling and efficacy of drugs against the viruses causing infectious diseases was also discussed.

There has been a tremendous improvement in the quality and complexity of the multi-organs-on-chip for simulating human physiology. The microfluidic devices for developing vascularized organs have also been demonstrated. These approaches offer accuracy and support replacement of existing models, reduction in the number of animals, and refinement of existing models for a deeper understanding of biological processes at the tissue and organ levels. Though in vivo models are known to provide cross-organ communication, the interpretation and analysis at the molecular level was not possible, which could be easily achieved by using these microfluidic multi-organs-on-chip devices.

The multi-organs-on-chip currently remains a potential alternative and complementary approach to the conventional models. These devices are a huge success academically, but their translation remains quite challenging. Although there is great scope for these devices and could reduce the number of in vivo studies significantly, more validations are required showing the efficacy and superiority of these devices over widely accepted in vivo studies. The design and fabrication of these devices is also challenging, which need to be considered for their translation. The designing and fabrication of these devices should be simple, the devices should be easy to use and handle, the devices should be reconfigured and can be used multiple times, and they should be fully automated for their complete acceptance and use in the healthcare industry.

References

1. Baker EJ, Beck NA, Berg EL, Clayton-Jeter HD, Chandrasekera PC, Curley JL, Donzanti BA, Ewart LC, Gunther JM, Kenna JG (2019) Advancing nonclinical innovation and safety in pharmaceutical testing. Drug Discov Today 24(2):624–628

2. Sato T, Clevers H (2013) Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. Science 340(6137):1190–1194
3. Picollet-D'hahan N, Zuchowska A, Lemeunier I, Le Gac S (2021) Multiorgan-on-a-chip: a systemic approach to model and decipher inter-organ communication. Trends Biotechnol. https://doi.org/10.1016/j.tibtech.2020.11.014
4. Ingber DE (2020) Is it time for reviewer 3 to request human organ chip experiments instead of animal validation studies? Adv Sci 7(22):2002030
5. Liu F, Huang J, Ning B, Liu Z, Chen S, Zhao W (2016) Drug discovery via human-derived stem cell organoids. Front Pharmacol 7:334
6. Farkhondeh A, Li R, Gorshkov K, Chen KG, Might M, Rodems S, Lo DC, Zheng W (2019) Induced pluripotent stem cells for neural drug discovery. Drug Discov Today 24(4):992–999
7. Yin Y-B, de Jonge HR, Wu X, Yin Y-L (2019) Mini-gut: a promising model for drug development. Drug Discov Today 24(9):1784–1794
8. Low LA, Mummery C, Berridge BR, Austin CP, Tagle DA (2021) Organs-on-chips: into the next decade. Nat Rev Drug Discov 20(5):345–361
9. Sung JH, Shuler ML (2009) A micro cell culture analog (μCCA) with 3-D hydrogel culture of multiple cell lines to assess metabolism-dependent cytotoxicity of anti-cancer drugs. Lab Chip 9(10):1385–1394
10. Esch MB, Smith AS, Prot J-M, Oleaga C, Hickman JJ, Shuler ML (2014) How multi-organ microdevices can help foster drug development. Adv Drug Deliv Rev 69:158–169
11. Li AP (2009) The use of the integrated discrete multiple organ co-culture (IdMOC®) system for the evaluation of multiple organ toxicity. Altern Lab Anim 37(4):377–385
12. Greek R, Menache A (2013) Systematic reviews of animal models: methodology versus epistemology. Int J Med Sci 10(3):206
13. Rajan SAP, Aleman J, Wan M, Zarandi NP, Nzou G, Murphy S, Bishop CE, Sadri-Ardekani H, Shupe T, Atala A (2020) Probing prodrug metabolism and reciprocal toxicity with an integrated and humanized multi-tissue organ-on-a-chip platform. Acta Biomater 106:124–135
14. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71(3):209–249
15. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F (2021) Cancer statistics for the year 2020: an overview. Int J Cancer. https://doi.org/10.1002/ijc.33588
16. Liu X, Fang J, Huang S, Wu X, Xie X, Wang J, Liu F, Zhang M, Peng Z, Hu N (2021) Tumor-on-a-chip: from bioinspired design to biomedical application. Microsyst Nanoeng 7(1):1–23
17. Tatosian DA, Shuler ML (2009) A novel system for evaluation of drug mixtures for potential efficacy in treating multidrug resistant cancers. Biotechnol Bioeng 103(1):187–198
18. Cho S, Yoon J-Y (2017) Organ-on-a-chip for assessing environmental toxicants. Curr Opin Biotechnol 45:34–42
19. Satoh T, Sugiura S, Shin K, Onuki-Nagasaki R, Ishida S, Kikuchi K, Kakiki M, Kanamori T (2018) A multi-throughput multi-organ-on-a-chip system on a plate formatted pneumatic pressure-driven medium circulation platform. Lab Chip 18(1):115–125
20. Virumbrales-Muñoz M, Ayuso JM, Olave M, Monge R, de Miguel D, Martínez-Lostao L, Le Gac S, Doblare M, Ochoa I, Fernandez LJ (2017) Multiwell capillarity-based microfluidic device for the study of 3D tumour tissue-2D endothelium interactions and drug screening in co-culture models. Sci Rep 7(1):1–15
21. McAleer CW, Long CJ, Elbrecht D, Sasserath T, Bridges LR, Rumsey JW, Martin C, Schnepper M, Wang Y, Schuler F (2019) Multi-organ system for the evaluation of efficacy and off-target toxicity of anticancer therapeutics. Sci Transl Med 11(497):eaav1386
22. Islami F, Miller KD, Siegel RL, Zheng Z, Zhao J, Han X, Ma J, Jemal A, Yabroff KR (2019) National and state estimates of lost earnings from cancer deaths in the United States. JAMA Oncol 5(9):e191460–e191460

23. Ong LJY, Ching T, Chong LH, Arora S, Li H, Hashimoto M, DasGupta R, Yuen PK, Toh Y-C (2019) Self-aligning Tetris-like (TILE) modular microfluidic platform for mimicking multi-organ interactions. Lab Chip 19(13):2178–2191
24. Xu Z, Li E, Guo Z, Yu R, Hao H, Xu Y, Sun Z, Li X, Lyu J, Wang Q (2016) Design and construction of a multi-organ microfluidic chip mimicking the in vivo microenvironment of lung cancer metastasis. ACS Appl Mater Interfaces 8(39):25840–25847
25. Kong J, Luo Y, Jin D, An F, Zhang W, Liu L, Li J, Fang S, Li X, Yang X (2016) A novel microfluidic model can mimic organ-specific metastasis of circulating tumor cells. Oncotarget 7(48):78421
26. Toh Y-C, Raja A, Yu H, Van Noort D (2018) A 3D microfluidic model to recapitulate cancer cell migration and invasion. Bioengineering 5(2):29
27. Aleman J, Skardal A (2019) A multi-site metastasis-on-a-chip microphysiological system for assessing metastatic preference of cancer cells. Biotechnol Bioeng 116(4):936–944
28. Mao M, Bei HP, Lam CH, Chen P, Wang S, Chen Y, He J, Zhao X (2020) Human-on-leaf-Chip: a biomimetic vascular system integrated with chamber-specific organs. Small 16(22):2000546
29. Wang Y, Wang P, Qin J (2021) Microfluidic organs-on-a-Chip for modeling human infectious diseases. Acc Chem Res 54(18):3550–3562
30. Thacker VV, Dhar N, Sharma K, Barrile R, Karalis K, McKinney JD (2020) A lung-on-chip model reveals an essential role for alveolar epithelial cells in controlling bacterial growth during early tuberculosis. bioRxiv
31. Tang H, Abouleila Y, Si L, Ortega-Prieto AM, Mummery CL, Ingber DE, Mashaghi A (2020) Human organs-on-chips for virology. Trends Microbiol. https://doi.org/10.1016/j.tim.2020.06.005
32. Martinez AW, Phillips ST, Whitesides GM, Carrilho E (2010) Diagnostics for the developing world: microfluidic paper-based analytical devices. ACS Publications, Washington, DC
33. Villenave R, Wales SQ, Hamkins-Indik T, Papafragkou E, Weaver JC, Ferrante TC, Bahinski A, Elkins CA, Kulka M, Ingber DE (2017) Human gut-on-a-chip supports polarized infection of coxsackie B1 virus in vitro. PLoS One 12(2):e0169412
34. Shrestha J, Razavi Bazaz S, Aboulkheyr Es H, Yaghobian Azari D, Thierry B, Ebrahimi Warkiani M, Ghadiri M (2020) Lung-on-a-chip: the future of respiratory disease models and pharmacological studies. Crit Rev Biotechnol 40(2):213–230

Prospects of Medical-Device-on-a-Chip

26

K. B. Megha, A. Arathi, X. Joseph, and P. V. Mohanan

Abstract

Microfluidic technology is a highly advanced research platform that comes within the Micro-Electro-Mechanical-Systems (MEMS) that is concerned with controlling the flow of fluids that can be measured in micro-, nano-, or pico-litre quantities. This technique provides cost-effective and disposable tools for rapid diagnosis and point-of-care device application. The development of a medical-device-on-a-chip by utilizing microfluidic technology could save time and cost over conventional testing methods and replace a macroscale device. The technique would potentially reduce the financial and ethical burdens in the biomedical field. This chapter provides the importance of microfluidics and their application in developing medical-devices-on-a-chip.

Keywords

Microfluidics · Point-of-care · Medical-device-on-a-chip · Biomedical

26.1 Introduction

For the past centuries, by utilizing high resources, new advanced technologies have improved the quality and betterment of human's health. This has led to progress in discovering contagious pathogens, antibiotics, and vaccines, which are some prominent examples. To date, medical technology shows outstanding advances, incredibly advanced brain surgery, development of artificial joints, heart and lung machines are

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only a few examples. The medical products regularly used in healthcare sectors are medications, vaccines, and medical devices. Medical devices have become an essential part of the healthcare sector, which helped reduce functional disabilities among people by early and quick diagnosis and treatment.

According to the Global Harmonization Task Force (GHTF), a medical device can be any apparatus, instrument, machine, implant, in vitro solution, software, or material intended to be used for prevention, diagnosis, and monitoring and treatment/mitigation of disease. It should also support or sustain life, providing the communication or information for medical/diagnostic determinations derived from the human body [1]. The crucial factors that depend on the enhanced access of medical devices include the 'supply, regulation and innovation'. The four critical aspects related to the appropriate usage of medical devices include the availability of the device in the market; accessibility to obtain relevant and good quality health aids; and the appropriate device that can be well maintained and utilized at an affordable price. Continuous research in medical devices is a requisite for disease and dysfunction to focus on most types of illnesses and functional disabilities currently affecting people globally. Many people live with disabilities due to trauma/injury, chronic disease conditions, infectious disease, malnutrition, and violence. Inappropriately, many medical device sectors emphasize their profit rather than necessity. Identifying the gap between the medical devices and the clinical requirement can provide a possible solution to improve worldwide access to suitable medical devices.

Microfluidics opens a vast opportunity to the medical sector to create suitable medical devices. The microfluidic system can be utilized to switch pico-litre to microlitre volume in possible ways than other methods for fluid handling. The technology is used to fabricate devices with microminiaturized sizes consisting of chambers and channels with 1 mm or less size to regulate fluids' flow behaviour [2]. The microfluidic devices cultured with human cells have the capability to mimic the physiologic environment of tissues and organs in a three-dimensional microenvironment and help to understand the underlying mechanisms of pathological conditions, drug interaction, and discovery. Microfluidic devices have the characteristic benefit of rapid analysis, high throughput screening using limited consumption of chemicals, highly biocompatible, cost-effective, and automated in biomedical, biological, and chemical studies [3–5]. The book chapter is intended to understand the different aspects of medical devices on a chip.

26.2 Development Pathway and Design Considerations for Medical Devices

Designing and developing a medical device are the most critical stages that finally determine the device's success. The lack of well-defined and designed medical devices cannot meet the desired function and benefits that will fail the regulatory requirements. The primary or the initial stage is to identify the medical need and market necessity. The next stage determines the device classification based on the

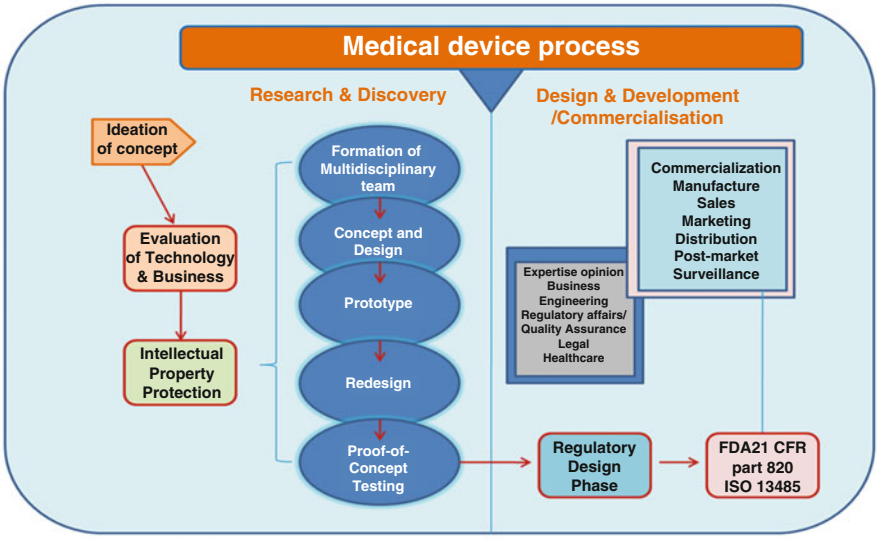

Fig. 26.1 Product conceptualization and discovery stage

risk connected with the use and obligatory by law. It is mandatory to follow procedures of the FDA and Intellectual Property Right (IPR).

The successful completion of the discovery stage should include a strong expert team from the following areas of engineering and design, clinical and scientific knowledge of the medical industry, regulatory affairs, quality assurance and intellectual property act. This stage coordinates with the initial 'idea' in a designed, prototyped, proof of concept testing, and recurrence-determined redesign. After successfully completing the product conceptualization and discovery stage, later proceed for FDA approval and commercialization (Fig. 26.1).

26.3 Microfluidic Technology for Biomedical Application: Fundamentals

Microfluidics is the branch of Micro-Electro-Mechanical Systems (MEMS) that regulates fluid flow measured in micro, nano- or pico-litre volume. The fluid can be either liquid or gaseous substance or a combination of the two. The flow travels through microscale channels, pumps, filters, and valves. These devices are fabricated on silicon or glass as well as on organic materials like plastics and polymers using photolithographic and etching processes modified from the semiconductor industry [6, 7]. Microfluidic devices have a large surface-to-volume ratio that necessitates only a low amount of sample/reagent for handling and processing. Additionally, due to their fast reaction time and easiness of automation, the devices are well suited for various applications in biomedical engineering.

Microfluidics is a widely accepted technique for total analysis systems or lab-on-chip devices [7, 8], mainly utilized for drug screening and microarray development [9]. Over 20 years of intense research, the technology is rapidly evolving with maximum progress, and within the near future, customized microfluidic devices addressing specific clinical, pharmacological, biotechnological demands can be accomplished. Point-of-care diagnosis is one of the most hopeful applications of microfluidics in biomedical engineering. The isolation of targeted cells from biological samples is highly prioritized during sample preparation. The separation of the cells in a fluidic suspension mainly depends on the size, density, electrical charge, surface antigens, and light scattering properties. Separating cells based on these criteria can necessitate sophisticated technologies and specialized equipment. Centrifugation, fluorescence-activated cell sorting (FACS), affinity, and magnetic separation are examples of such techniques.

26.4 Actuation Mechanisms for Microfluidic Medical Devices

The dominance of surface and viscous forces at smaller scales makes actuation and manipulation of fluids and particles in a microfluidic device a significant task, especially when integrated with a portable handheld device for point-of-care diagnostics and biosensing purposes. The actuating and manipulation of fluids and particles at microscale dimensions is very challenging, mainly due to the surface-area-to-volume ratio as the size of the characteristic system is lowered, which gets reflected in the elevated dominance of surface and viscous force in retarding fluid motion. It is generally represented as small Reynolds numbers ($\mathrm{Re} \equiv \rho UL/\mu \leq 1$) where ρ and μ are the density and viscosity of the fluid and U, and L represents the velocity and length scale in the microfluidic system, respectively. The property of laminarity of the flow is also an inherent factor among these low Re systems, posing additional fluid mixing/blending issues, particularly in diffusion-limited systems. An external syringe pump is a pre-requisite and commonly utilized method to propagate flow and mixing in microfluidic systems. Most medical testing includes molecular and bioparticle manipulation, requiring additional microfluidic competencies for rapid and sensitive preconcentration, sorting, and detection. The mechanisms for microfluidic actuation come within the mechanical and non-mechanical actuation categories illustrated in Fig. 26.2.

26.4.1 Mechanical Actuation Mechanisms

26.4.1.1 Piezoelectric

It comprises a disc or stack of piezoelectric (diaphragm) that deforms when exposed to an electric field to persuade peristaltic fluid motion laterally to the length of the channel [10, 11].

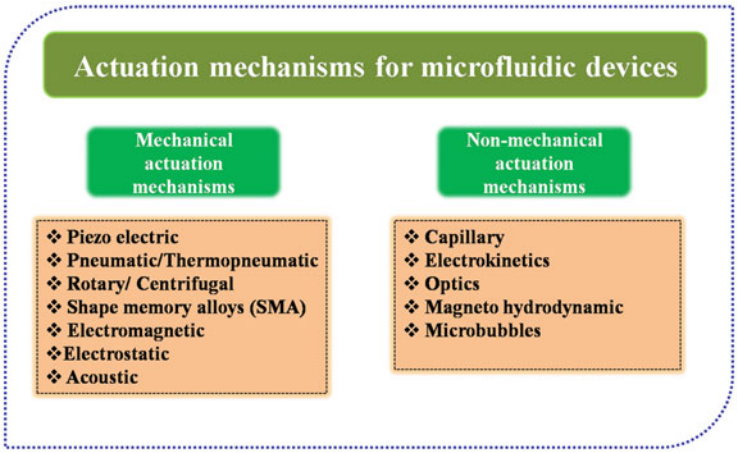

Fig. 26.2 Actuation mechanism for microfluidic devices

26.4.1.2 Pneumatic/Thermopneumatic

The diaphragm is made to actuate and relax by air to create a pressure variation to pump the fluid, which is often combined with diffusers. Both heated and cooled air is used in thermopneumatic versions [12–14].

26.4.1.3 Rotary/Centrifugal

The valves, channels, and reservoirs are patterned on a compact disc (CD). The centrifugal force provides fluid actuation upon rotation using a micromotor facility. The thermopneumatic combination can provide bidirectional pumping by heating the reservoir [15, 16].

26.4.1.4 Shape-Memory Alloys (SMAs)

They are thin films. They are recently advanced as wires that are used as valves, pumps, latches, and multiplexers that have the ability to apply large strains on the elastomers such as PDMS in the electric field [17, 18].

26.4.1.5 Electromagnetic

By applying an oscillating magnetic field, the fluid actuation is achieved due to vibration, which is generated by the use of magnetic elements that are embedded in the soft polymeric surfaces [19, 20].

26.4.1.6 Electrostatic

The force between oppositely charged plates that are driven when the proper voltage is applied results in the deflection of a soft membrane known as the coulombic attraction. This deflected membrane returns to its normal position upon relaxation of the applied field [21, 22].

26.4.1.7 Acoustic

- Flexural waves: Bulk vibrations can cause an acoustic field in a thin piezoelectric film that in turn make the fluid move, i.e. acoustic streaming [23–25].
- Bubble streaming: Acoustic streaming is operated by the action of bubbles that are adhered to the channel by a piezoelectric transducer. It is mainly used for fluid mixing, particle sorting, and trapping [26, 27].
- SAW (Surface acoustic waves): The electromechanical surface waves of mega-hertz frequency can directly generate an acoustic force on the particles, leading to fluid flow (acoustic streaming) and thereby manipulating biomolecule and micro/nanoparticles [28].

26.4.2 Non-mechanical Actuation Mechanisms

26.4.2.1 Capillary

- Pressure gradient: The pressure difference across an interface can induce flow that creates wetting fluids along the channels or paper built. It produces a passive actuation system without the requirement of any pump [29].
- Surface tension gradient: The interfacial flow induced by thermal (thermocapillary), electrical (electrocapillary) or optical (optocapillary) gradients or by chemical concentration [30, 31].

26.4.2.2 Electrokinetics

- Electro-osmosis: It is attained by applying an external electric field that controls the motion of the aqueous solution through a fixed solid boundary [32].
- Electrophoresis: The movement of charged particles or ions in a fluid at a stationary phase attained by applying an external electric field [33].
- Dielectrophoresis: The movement of dielectric particles suspended in a medium with the aid of a non-uniform electric field [34].
- Electrowetting: To regulate the drop/film wettability by applying an electric field to generate Maxwell force (contact line) or Maxwell pressure (fluid interface) based on the electrode configuration [35].

26.4.2.3 Optics

- Laser microfluidic actuation: Due to the difference in refractive index, the momentum carried by the propagating light causes a radiation pressure at the fluid interface, resulting in jetting or deformation at the interface. Furthermore, the localization of the thermocapillary forces is induced by the laser beam, resulting in fluid flow [36–38].
- Optical tweezers: The optical gradient within the tightly focused laser beam can trap and transport dielectric particles [39].

26.4.2.4 Magnetohydrodynamic

To pump conducting fluids perpendicular to both magnetic and electric fields using Lorentz force [40, 41].

26.4.2.5 Microbubbles

The bubble oscillation method is generally used to generate pumping in the channels either incorporating electrochemically or thermally.

The fluid actuation and manipulation of particles can create revolutionary, innovative explanations beyond the expectation in both biological and chemical applications. The technology can provide much faster, economical, significantly much less reagent volume for specific techniques and methods, e.g. Polymerase Chain Reaction, proteomics, point-of-care diagnostics are some of them [42]. But some limitations are yet to be cleared, such as the miniaturization of the device, i.e. difficulty in scaling down, integrating efficient fluid actuation and particle manipulation. Aside from miniaturization, actuation efficiency is a significant hurdle that has yet to be overcome. These limitations can be overcome with continuous improvements in microfluid actuation research and development.

26.5 Digital Technologies for Medical Devices

Droplet-based digital microfluidics is becoming increasingly imperative in the field of chemical, biological, and health science. Such systems are characterized by their excessive precision and exceptional reagent economy. The continuous flow topologies with in-line, one-dimensional (1D) flow channels are currently used for microfluidic microdrop motion. The easiness of use of this 1-D fluid flow system allows fabrication of the standard device using wet etching and micro-milling techniques [43]. The potentiality of these 1-D microfluidic devices has been astounding, with the best result coming from fluid control applications that need filtering [44] or mixing [45]. The fluid flow through a 1-dimensional flow channel is prone to obstruction and clogs, and such failures can be disastrous. A permanent solution to topologies can provide few possibilities for rerouting and adapting. The terms reconfigurability/fault control are often used interchangeably.

The 2-D plane microdrop motion offers more opportunities for adaptations through real-time fault control, in addition to improved potentials for parallel on-chip microdrop mixing/splitting algorithms in bio- or chemical reactors [46]. A generalized microdrop motion platform with the capability of microdrop motion in a 2-D plane is used in a digital microfluidic configuration. In digitalized microfluidics, microdrop motion is not based on pressure and is not required to limit motion to 1-D flow channels. In its place, localized voltage impulses across the 2-D plane are used to create microdrop motion. The user-controlled voltage distributions are carried out to perform fluid processing tasks, with voltage signals providing the modifications [47]. The 2-D digital microfluidic devices can address expanding needs for minimal fluid volume, high-sensitivity, and parallel high throughput on-chip analysis procedures. There is a great requisite for handling a higher volume of fluid using digital microfluidic microdrop generation/extraction. Microdrop motion scalability is a foremost concern in any 2D digital microfluidics system. Microdrop motion is dynamic, and it necessitates proper temporal synchronization and voltage localization from various system inputs to cause interfacial surface tension changes and

microdrop motion [48]. This case is more challenging when a device has more system instructions and detailed spatial resolutions. Another issue is that fluid sampling sensitivity is reduced while focussing towards a smaller scale and minimal reagent volume.

The droplet-based digital microfluidic technology is advancing rapidly. The accurate control and minimal reagent consumption have made these technologies excel in the biomedical field. By the application of microfluidic technology, the processes such as immunoassays [49], DNA ligations [50], and pyrosequencing [51], enzymatic study [52], and bio-analytical mass spectrometry [53] are highly benefited. Further research is needed on the challenges for the above-mentioned digital microfluidic performance. The initial challenge to be addressed is the reagent's evaporation that directly influences their minimal volume consumption. To control the external contamination and the cross-contamination between internal fluid samples is really a practical concern. To overcome such complications, proper film layers and wash operations can be incorporated to control the contamination [54]. To maintain the microdrops is becoming a significant issue of increased concern when fine resolution and a large number of droplets are necessary and can be sorted out with the incorporation of mixing, sorting, and splitting [55].

26.6 Medical Devices-on-a-Chip

The economical and ethical considerations of animal experiments and clinical trials limit the development of medical device technology. Organ-on-a-chip systems are being shaped up to speed up drug development. Still, the application of this technology in the creation and testing of medical devices has received little acknowledgement or funding. The idea of medical-device-on-a-chip (MDoC) highlights the potential application of microfluidic high-throughput technologies for delivering significant time and cost reduction than the conventional testing. Evaluating an entire macroscale device, an MDoC can mimic the biological function in a physiome linked to medical device use and test interactions with device components [56].

Even though maximum medical devices are too large to get aligned on a microchip, the same notion that underpins organ-on-a-chip technologies replicating or remodelling a functional unit of an organ can also be applied to medical devices. It is typically easy to integrate a functional unit of a medical device with a corresponding tissue or organ model that can fit on a chip. Several potentially similar MDoC models exist for each prevailing organ-on-a-chip model produced for medication or disease testing to be adapted for testing medical equipment (Table 26.1).

MDoCs could be useful in the toxicological assessment of novel materials and device drug or device-biologic combinations for which there is limited data availability. It has been proposed for the toxicological safety testing of nanomaterials using advanced tissue models [57]. The advantages of MDoCs in terms of speed, control, reproducibility, and cost, mainly when human-on-a-chip testing is used to provide toxicological data [58], could provide a competitive edge for completing the '3Rs'. One of the significant advantages of the MDoC model is the ability to sum up

Table 26.1 Examples of MDoC and their corresponding medical devices

Organ-on-a-chip	Examples of MDoC applications	Corresponding medical devices
Body (multi-organ)	Systemic toxicity	All appropriate devices
Bone	Osseointegration	Orthopaedic implants
Bladder	Infection pathogenesis	Urinary catheters
Brain	Reliability	Neural implants
Eye	Ocular irritation or sensitization, and cleaning performance	Contact lenses and care solutions
Gut	Cancer identification and treatment	Nanoparticles for theranostic application
Kidney	Excretion	Any appropriate device
Lung	Bacterial colonization	Ventilator for endotracheal tubes
Mouth	Tubule occlusion/remineralization	Dental implants, materials and solutions
Muscle	Muscle rehabilitation effectiveness	Electric muscle stimulator
Reproductive organ	Developmental toxicity	Any appropriate device
Skin	Dermal sensitization	Wound dressing
Vasculature	Kinetics of drug release	Stents for drug-elution
Heart	Blood biocompatibility	Ventricular assist devices
Prostate	Drug release kinetics	Prostate implants
Liver	Metabolism/detoxification	Any appropriate device

biological function by more practical simulating in vivo microenvironments. It is possible to grow tissue-culture models in a microchip, and microfluidics' continuous perfusion can provide nutrients needed for growth and maintenance during the testing procedure.

26.6.1 Drug Discovery

The progression of developing new therapeutics for pathophysiological conditions begins with identifying a specific target molecule [59]. The target molecule is determined with the help of computational biology or by the experimental method, followed by validation in a complex cell or animal model studies. Further clinical trials followed with final FDA approval takes 10–15 years of time and cost [60]. The final scrutinization of a successful therapeutic agent is a result of the incompetent procedures in the conventional/current drug discovery and developmental processes. The continuous advancement provided by the microstructured platform supports the process of drug discovery more resourceful and prompt with relevant information on biological targets [61]. The high-throughput microfluidic devices have dramatically reduced the processing time generally required over other conventional methods.

The highly improved isolation techniques and identification of the pharmacological response have developed a newer approach for drug target identification and reduction in usage of living systems [62]. Commonly, an ion channel, cellular receptor, nucleic acids, enzymes, polysaccharides, and lipids are the chemical structures capable of interacting with the drug molecules [63]. The most important drug targets are kinases, phosphatases, proteases, and G-protein-coupled receptors. Ion channel proteins are another promising target in the drug discovery process, as they have been linked to various cardiovascular and neurological diseases [64].

Most of the drug targets are part of the lipid bilayer structure of the cell membrane, and their function mainly depends on the integrity of the membrane. The proteins get denatured after detaching from the membrane, hence they must be incorporated within the membrane throughout the analytical procedure [65]. For the successful fabrication of lipid bilayer membrane, microfluidic technology played a pivotal role in simulating a natural environment for drug target study [66]. The interactions between receptors and their ligands provide insight into disease progression and lead to discovering drugs based on their selective interaction with the target receptor [67]. Thus for ligand-binding studies, microfluidics is beneficial by providing minimal interaction time, enhanced sensitivity and aids in the separation of both complexed and uncomplexed molecules [68].

26.6.2 Cellular Analysis and Tissue Engineering

The biological cells must sustain their functionality in vitro for accurate cell-based procedures. In order to construct systems for long term cell survival, a thorough understanding of cell properties and factors that can lead to cellular harm in microfluidics is a requisite. Despite prokaryotic or eukaryotic organisms, the biological cells are the functional unit of all living organisms [69]. They are typically structured as an internal fluid compartment (cytoplasm) interspersed with microscopic organelles (cytoskeleton, mitochondria, ribosomes, golgi apparatus, etc.) with various functional responsibilities that keep both internal and external environments running well. Different biological processes influence the external environment (DNA replication, protein synthesis, and biochemical signalling). The membrane-spanning mechanosensors detect disturbances in the cell wall/plasma membrane, activating the cell wall integrity (CWI) signal transduction pathway, inducing gene expression for products involved in the cell wall assembly and remodelling [70].

The microenvironment condition such as the potassium ion concentration, negatively charged proteins, and phosphate ions is higher within the cells than outside, whereas sodium and chlorine ions are more plentiful outside the cell. This creates an electrochemical gradient that is continuously synchronized by the ion channels to restore homeostasis. So any change in the osmolarity and salinity detected by the sensors has initiated signalling pathways that altered the rate of protein synthesis and the electrolyte concentration-response [71]. It was also observed that the extreme osmotic stresses on the kidney mammalian cells had reasoned genomic damage and cell cycle interference. ‘Osmotic shock’ can happen when the cells are changed from

their usual environment and positioned in the microfluidic system with insufficient media formulations. In the microfluidic system, mixing and dilutions of fluids become more multifaceted, and their variation in hydrodynamic resistance due to viscosity alteration is evident [72]. Biological cells are dielectric materials with electrophysiological, biophysical, and optical properties that differ from one another. By utilizing these features, one can attain high accuracy and precision in manipulating and selecting particles. In both batch- or continuous-flow processes, manipulation processes include characterization, sorting, separation, trapping, patterning, concentrating, or focusing cells are all possible. A highly efficient microfluidic flow cytometric device that focuses, counts, detects, or sorts cells on a single chip can be advantageous compared to the more complex, expensive, and bulkier FACS flow cytometers.

Microfluidic device platforms can provide the necessary insights to overcome barriers to clinical translation. That is, optimizing soluble factors to improve cell expansion and differentiation outcomes that affect the pore architecture and surface engineering on the scaffold colonization and the biophysical needs of cells when creating three-dimensional artificial vascular pedicles for better scaffold vascularization post-implantation. The scaffolding material impacts colonization by changing specific cell-material interactions (for example, those mediated by integrins), which influences cell adhesion and motility [73]. Effective cell seeding and scaffold colonization remain a significant problem. Existing seeding procedures must be optimized for each case because of limits in our knowledge and understanding of the appropriate parameter space. One of the most prominent and challenging aims is to engineer large 3D artificial structures by forming a microcirculatory network. The most common method to fabricate microcirculatory devices comprises microfabrication.

A well-structured and prominent vascular network is essential for a microcirculatory network. One of the probable difficulties faced was the mismatch between the flow and cells that created the endothelization in the initial stages of microdevice development [74, 75]. Moreover, the stiffness of the material (PDMS-580 kPa) [76] compared to natural tissues [77] hinders angiogenesis. So these microdevice limits the tissue-engineered constructs for implantation. This eventually leads to the development of more biocompatible materials by exploring native extracellular matrix proteins and hydrogels for microfabrication. Microfluidic devices have only recently been used to convert bulk hydrogel systems into cell-laden microgel particles, offering considerable benefits such as uniform size, regulated physical and chemical characteristics, degradation rate, and encapsulated cell density. Some examples of materials to make hydrogel-based microdevice includes collagen [78, 79], alginate [79–81], agarose [82], and poly(ethylene glycol) [83]. With the rapid advancement in the fabrication of microdevices from various materials with high accuracy and fidelity, a highly controlled device platform could ultimately control tissue repair and remodelling.

26.6.3 Single-Cell Trapping and Micro-robotic Injection

A single cell is trapped or immobilized into a regular pattern for cell manipulation procedure that has wide application in studies based on a single cell, such as molecule/drug screening [84], cell pairing and fusion [85], and DNA damage investigation [86]. With the more significant advancement in microfluidic technology, various techniques are developed for cell trapping based on the different mechanisms as surface chemistry [87], optical tweezers [88], dielectrophoresis [89], magnetic and ultrasonic trapping [90, 91], and by mechanical constrains [92]. In addition to the applications described above, single-cell trapping and immobilization is the most capable and efficient technique for microinjection. That is to introduce the target materials into cells for molecular genetics, screening and reproductive studies. The progress in the microfluidic has the capability to rapidly immobilize many cells in a particular model to make it possible for both manual and robotic microinjection.

Only the mechanical confinements provide sufficient immobilization force for microinjection. The suitable structure for mechanical confinements includes the microwells [93], hydrodynamic traps [94], and vacuum-based form [95]. However, microwells cannot provide a secure platform for cell penetration, as the cells entrapped in the wells can slightly move [93]. Hydrodynamic traps provide a closed microchannel, consequently preventing the injection of cells [92]. At the same time, the vacuum-based type immobilizes individual cells in an array of micrometre-sized holes coupled to a vacuum chamber. The differential interference contrast (DIC) microscopy is generally employed for the cell imaging of microinjection. Mostly glass is chosen as the construct material for cell immobilization devices for microinjection applications [96]. Reliable automated microinjection reduces cell-holding devices' adverse effects on post-injection for embryonic development. The immobilized zygotes were introduced with the aid of a robotic injection system [97]. A microrobot manages the injection micropipette to penetrate the immobilized zygote diagonally and deliver materials into the cytoplasm.

26.6.4 Stem Cell Analysis

Stem cells can differentiate into almost any type of cell in the body. However, the limited insight into stem cell biology and its differentiation mechanism impede the translation of stem cells from the bench to the bedside. The advancement of microfluidic technology offered a powerful tool for understanding stem cell mechanisms. Due to the unlimited regeneration property of the stem cells, they can be utilized to provide better treatment strategies using stem cell-based regenerative medicine. So, with the aid of microfluidic technology, it can promote advancement in understanding towards stem cell analysis and their future possibilities. Mainly two types of stem cells are found in mammals; embryonic stem cells derived from the inner cell mass of blastocysts and adult stem cells found in various tissues and bone marrow. Regardless of the origin and potency of embryonic and adult stem

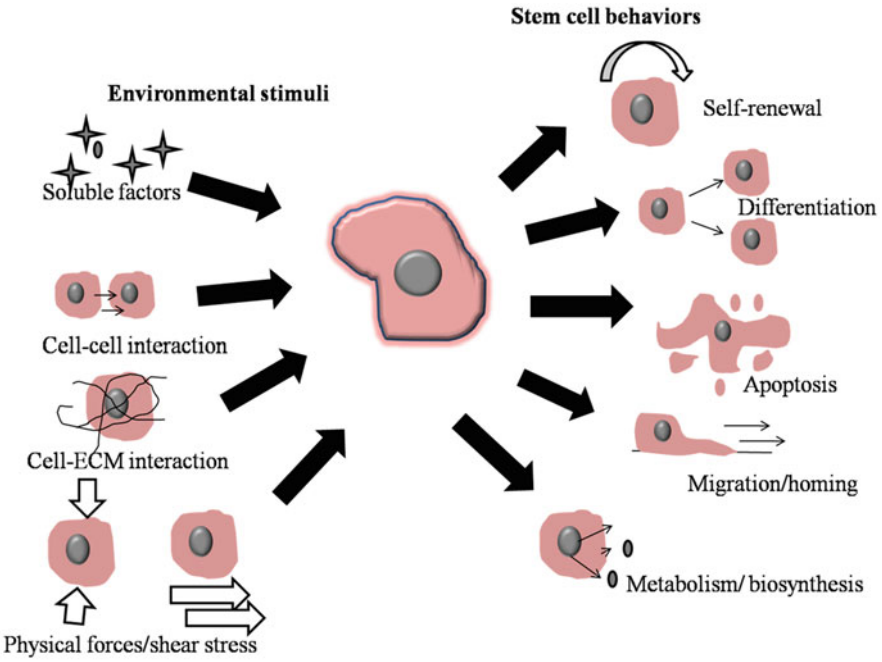

Fig. 26.3 Microfluidic-based stem cell culture platform to provide in vivo culture condition

cells, both hold an exceptional potential to impact human health. The induced pluripotent stem cells (iPSC) originated from the adult liver, stomach, and skin cells can differentiate into all of the adult body cells [98]. The stem cells have gained much attraction as therapeutic agents in regenerative medicine by replacing the damaged cells, tissues, or organs to reinstate the regular function [99]. Hematopoietic stem cells (HSC) have improved the treatment of autoimmune diseases [100, 101], and mesenchymal stem cells (MSC) are used as a regenerative agent for treating damaged heart tissue, diabetes, liver, bone, cartilage and spinal cord injury, vascular diseases, autoimmune diseases, etc. [102]. The lack of knowledge regarding stem cell biology hinders their vital properties that can help overcome many prevailing adverse health conditions (Fig. 26.3).

The development of a new culture platform is a requisite to understand the essential role played by each factor or signal that controls the microenvironment of the stem cell niche. These cross-talks indicate the appropriate cellular response that makes them undergo differentiation, self-renewal, or apoptosis. Miniaturized devices, also known as the 'micro total analysis system' (μTAS), provide a better cell analysis platform. These miniaturized devices offer many advantages, like reduction in cell number and reagent supply, control over signal gradients, flow and time, high throughput, complete automation. Thus providing enormous opportunities to characterize, analyse, and manipulate cells. The microfluidic cell culture system provides spatial and temporal control over cell response and can be stimulated by mimicking

the complex extracellular matrix (ECM) within the fluidic channel [103, 104]. The cellular fate and functions of the stem cells are controlled by combined intrinsic programs and signals from the microenvironment. It is reported that hydrogels are commonly used to replicate the extracellular matrix in a microfluidic device to study interactions within the cells [105]. Mostly collagen, fibrin, alginate, gelatin, and agarose are used for cellular matrix [106].

Song and colleagues introduced the concept of droplet microfluidics [107] to address the concern with cross-contamination, interactions within the solute surface, reagent volume and continuous flow in long channel lengths in microfluidics. Moreover, protein and gene expression, cytotoxicity, cell proliferation and differentiation, enzyme kinetics, cell signalling have been achieved using the droplet microfluidics, not by continuous fluid device [108, 109]. Also, the introduction of microprinting has enabled a better understanding of the role of ECM components on stem cell phenotypes. Incorporation of microfluidic technology in stem cell research will provide a better platform for understanding the essential fundamental aspects as well for stem cell-derived clinical applications.

26.6.5 Paper-Based Microfluidic Devices

The easy-to-use paper testing devices for low cost have been used for many years for checking pH, pregnancy, and diabetes testing strips and have been commercially available for many years. Paper is a cellulose fibre that is abundant, affordable, and compatible with biological samples. With the advancement from microfluidics, researchers have improved these techniques and established microfluidic paper-based analytical devices (μPAD) [108]. Inexpensively, 'lab-on-paper' systems may perform complicated and precise qualitative and quantitative assays in a broader range of biochemicals. μPADs are intended to be a simple, portable, and mass-production solution that combines the advantages of both microfluidics and paper. μPADs rely on capillary action to circulate fluids. There are mainly two patterning strategies: One step is the selective hydrophobization of particular portions of the device while leaving the fluidic routes intact and hence hydrophilic, and two-step whole hydrophobization of certain areas for fluidic flow. Dehydrophobization is accomplished by removing or dissolving the previously deposited hydrophobic chemical, revealing the original hydrophilic paper beneath. The one-step technique preserves the fluidic channels and detection zones, leaving their physicochemical properties intact (e.g., the actual colour of paper, without leaving residues). For μPADs, a wide range of paper materials can be utilized as a substrate. The right one is chosen based on the field of its application and fabrication procedure. The most common type of paper used is cellulose paper as the material is inexpensive, naturally hydrophilic, and enables rapid liquid penetration. Hydrophobic nitrocellulose membranes, which are smooth and have a reasonably consistent pore size (0.45 m), are another essential type of paper substrate. They allow for a more steady and repeatable liquid flow. They have been utilized for antigen immobilization [109] and also in dot-immunobinding tests [110], since they display a high degree of

nonspecific binding towards biomolecules, making them appropriate for enzyme immobilization [111–113], proteins [114], cells [115], and DNA [116].

Colorimetric biochemical assays are well suited to μPADs, paper strip tests which are available commercially detect the analytes qualitatively by their indicative colour change [107]. When μPADs were first introduced, they were successfully used to detect glucose and protein using colorimetry at clinically relevant levels [117]. Unlike colorimetric assays, electrochemical detection (ECD) allows for exact quantification of analyte concentration. ECD is a redox-based approach that uses a three-electrode system; the working, counter, and reference electrodes. These electrodes can be screen-printed with conductive ink on paper-based electronics for a minimal cost. The working and counter electrodes are usually inked with carbon ink, whereas the reference electrode and pads are usually inked with silver/silver chloride ink [117, 118]. Dungchai et al. developed the first microfluidic paper-based electrochemical device (PED) for simultaneous quantification of glucose, lactate, and uric acid in biological samples [119].

Chemiluminescence (CL) is easy, sensitive, and may be done in the dark; thus, it is not affected by ambient light. Using the CL interaction between rhodamine derivatives and hydrogen peroxide, Yu et al. developed a μPAD for the simultaneous measurement of glucose and uric acid [120]. Electrochemiluminescent (ECL) detection refers to a CL reaction in which luminescence is induced and controlled by an electrical potential. This approach combines the benefits of CL, such as light emission independent of ambient light, with greater selectivity and a wider dynamic range due to better reaction control via electrochemistry [120–122]. ECL has the advantage of EC detection, such as high sensitivity and superior control over the reaction time and spatial position [123]. The two main types of μPADs are on-demand devices, which are empty generic platforms that require the user to introduce detection reagents before testing and ready to use devices, which are full devices with reactive sensing reagents already integrated in the detection zones. Microfluidics on paper has the potential to be a superior analytical platform for a wide range of applications in health diagnostics [124], environmental monitoring [125], and food quality testing [126] and forensics.

26.6.6 Viral Detection

Microfluidic approaches are becoming cost-effective and disposable diagnostic tools for viral infection. Microfluidic devices that are used to detect viruses selectively and sensitively based on immunoassays of virus-related antigens/antibodies, nucleic acid amplification, or flow cytometry. Rapid and accurate diagnostic tools are critical for detecting viruses and infections, administering appropriate therapy and averting disease outbreaks [127–129]. Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) are gold standard procedures for detecting viral infections [130, 131]. They're nevertheless time-consuming and labour-intensive and are usually coupled with high equipment and reagent costs [132]. According to WHO guidelines, to improve the health-related quality of life of global populations,

cost-effective, portable, disposable, and point-of-care diagnostic techniques are needed [133]. With their enhanced sensitivity and lower cost, microfluidic technologies hold a lot of potential for developing useful diagnostic tools that can overcome the limitations of traditional approaches [134]. Song et al. developed a microfluidic fluorescent immunoassay for quick, multiple HIV sample screening and confirmation [135].

Currently, the microfluidic technique is becoming an indispensable instrument for point-of-care viral diagnosis [136].

26.7 Conclusion and Future Perspectives

Microfluidic devices have exposed initial promise in all areas of drug discovery and development and the field of miniaturized diagnostic devices. The future of microfluidic devices for application in drug discovery appears bright, with a lot of research activity being focused on the development of miniaturized chips. Owing to the increased acceptance and potential benefits as economical alternatives to conventional bench-top macroscale equipment, it is imperative to develop integrated systems that are broadly accepted in all stages of drug discovery and development. For handling clinically significant samples, an intensive effort in combining multiple manipulation technologies is needed. Furthermore, microfluidics offers the prospect of hastening the identification of novel pathways and thus targeting through novel drug screening platforms.

Moreover, future trends in microfluidics-based stem cell research will help to examine stem cell differentiation and test the efficacy of engineered stem cells in an in vitro model that closely mimics human biology. Microfluidics possibly resolves to assess potential drugs for pregnant women as certain drugs are capable of being harmful to the foetus in the womb and provide a better tool to safely screen side effects of the drug candidates on embryonic stem cells. The drug companies could use this platform to further improve their stringency tests during drug development. Otherwise, microfluidics can be used to differentiate, analyse, and sort stem cells for cell therapy integration of multiple ‘organs on a chip’ to generate microfluidic systems that mimic the full range of physiology of humans useful for understanding normal biology, disease progression.

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References

1. Global Harmonization Task Force Study Group 1 (2005) Information document concerning the definition of the term “medical device”. The Global Harmonization Task Force (GHTF/SG1/N29R16:2005)
2. Mahesh K, Vaidya S (2017) Microfluidics: a boon for biological research. Curr Sci 112:2021–2028
3. Beebe DJ, Mensing GA, Walker GM (2002) Physics and applications of microfluidics in biology. Annu Rev Biomed Eng 4:261–286
4. Lagally ET, Mathies RA (2004) Integrated genetic analysis microsystems. J Phys D 37:R245–R261
5. Aurox P-A, Koç Y, de Mello AJ, Russell E (2004) Miniaturized total analysis systems for biological analysis. Lab Chip 4:534–546
6. Becker H, Locascio LE (2002) Polymer microfluidic devices. Talanta 56:267–287
7. Rivet C et al (2010) Microfluidics for medical diagnostics and biosensors. Chem Eng Sci. https://doi.org/10.1016/j.ces.2010.08.015
8. Prince M (2006) Smart microsystems for cell manipulations, PhD thesis, Aston University
9. Situma C, Hashimoto M, Scoper SA (2006) Merging microfluidics with microarray-based bioassays. Biomol Eng 23:213–231
10. Koch M, Harris N, Evans AGR, White NM, Brunnschweiler A (1998) A novel micromachined pump based on thick-fi lm piezoelectric actuation. Sens Actuators A 70:98–103
11. Schabmueller CGJ, Koch M, Mokhtari ME, Evans AGR, Brunnschweiler A, Sehr H (2002) Self-aligning gas/liquid micropump. J Micromech Microeng 12:420–424
12. Pol FCM, Lintel HTG, Elwenspoek M, Fluitman JHJ (1990) A thermopneumatic micropump based on micro-engineering techniques. Sens Actuators A Phys 21:198–202
13. Jeong OK, Yang SS (2000) Fabrication and test of a thermopneumatic micropump with a corrugated p + diaphragm. Sens Actuators A Phys 83:249–255
14. Kim J-H, Na K-H, Kang CJ, Kim Y-S (2005) A disposable thermopneumatic-actuated micropump stacked with PDMS layers and ITO-coated glass. Sens Actuators A Phys 120:365–369
15. Gorkin R III, Clime L, Madou M, Kido H (2010) Pneumatic pumping in centrifugal microfluidic platforms. Microfluid Nanofluid 9:541–549
16. Abi-Samra K, Clime L, Kong L, Gorkin R III, Kim TH, Cho YK, Madou M (2011) Thermo-pneumatic pumping in centrifugal microfluidic. Microfluid Nanofluid 11:643–652
17. Benard WL, Kahn H, Heuer AH, Huff MA (1998) Thin-fi lm shape-memory alloy actuated micropumps. J Microelectromech Syst 7:245–251
18. Xu D, Wang L, Ding G, Zhou Y, Yu A, Cai B (2001) Characteristics and fabrication of NiTi/Si diaphragm micropump. Sens Actuators A Phys 93:87–92
19. Al-Halhoulia AT, Kilanib MI, Üttgenb SBS (2010) Development of a novel electromagnetic pump for biomedical applications. Sens Actuators A Phys 162:172–176
20. Zhou Y, Amirouche F (2011) An electromagnetically-actuated all-PDMS valveless micropump for drug delivery. Micromachines 2:345–355
21. Bae B, Han J, Masel RI, Shannon MA (2007) A bidirectional electrostatic microvalve with microsecond switching performance. J Microelectromech Syst 16:1461–1471
22. Machauf A, Nemirovsky Y, Dinnar U (2005) A membrane micropump electrostatically actuated across the working fluid. J Micromech Microeng 15:2309–2316
23. Moroney RM, White RM, Howe RT (1991) Microtransport induced by ultrasonic lamb waves. Appl Phys Lett 59:774–776
24. Luginbuhl P, Collins SD, Racine G, Gretillat MA, Rooij NFD, Brooks KG, Setter N (1997) Microfabricated lamb wave device based on PZT sol–gel thin fi lm for mechanical transport of solid particles and liquids. J Microelectromech Syst 6:337–345
25. Meng AH, Nguyen N-T, White RM (2000) Focused flow micropump using ultrasonic flexural plate waves. Biomed Microdev 2:169–174

26. Wang C, Jalikop SV, Hilgenfeldt S (2012) Efficient manipulation of microparticles in bubble streaming flows. Biomicrofluidics 6:012801
27. Hashmi A, Yua G, Reilly-Collette M, Heiman G, Xu J (2012) Oscillating bubbles: a versatile tool for lab on a chip applications. Lab Chip 12:4216–4227. https://doi.org/10.1039/C2LC40424A
28. Friend JR, Yeo LY (2011) Microscale acoustofluidics: microfluidics driven via acoustics and ultrasonics. Rev Mod Phys 83:647–704
29. Ichikawa N, Hosokawa K, Maeda R (2004) Interface motion of capillary-driven flow in rectangular microchannel. J Colloid Interface Sci. https://doi.org/10.1016/j.jcis.2004.07.017
30. Darhuber AA, Troian SM (2005) Principles of micro fluidic actuation by modulation of surface stresses. Annu Rev Fluid Mech 37:425–455
31. Basu AS, Gianchandani YB (2008) Virtual microfluidic traps, fi lters, channels and pumps using Marangoni flows. J Micromech Microeng 18:115031
32. Lazar IM, Karger BL (2002) Multiple open-channel electroosmotic pumping system for microfluidic sample handling. Anal Chem 74:6259–6268
33. Kenyon SM, Meighan MM, Haye MA (2011) Recent developments in electrophoretic separations on microfluidic devices. Electrophoresis 32:482–493
34. Pethig R (2010) Dielectrophoresis: status of the theory, technology, and applications. Biomicrofluidics 4:022811
35. Wheeler AR (2008) Putting electrowetting to work. Science 322:539–540
36. Grigoriev RO (2005) Optical tweezers in colloid and interface science. Phys Fluids 17:033601
37. Baroud CN, Vincent MRS, Delville J-P (2007) An optical toolbox for total control of droplet microfluidics. Lab Chip 7:1029–1033
38. Dixit SS, Kim H, Vasilyev A, Eid A, Faris GW (2010) Light driven formation and rupture of droplet bilayers. Langmuir 26:6193–6200
39. Neale SL, Macdonald MP, Dholakia K, Krauss TF (2005) All-optical control of microfluidic components using form birefringence. Nat Mater 4:53–533
40. Lemoff AV, Lee AP (2000) An AC magnetohydrodynamic micropump. Sens Actuators B Chem 63:178–185
41. Eijel JCT, Dalton C, Hayden CJ, Burt JPH, Manz A (2003) A circular AC magnetohydrodynamic micropump for chromatographic applications. Sens Actuators B Chem 92:215–221
42. Robinson T, Dittrich PS (2013) Microfluidic technology for molecular diagnostics. Adv Biochem Eng Biotechnol 133:89–114. https://doi.org/10.1007/10_2012_139
43. Becker H, Locascio LE (2002) Polymer microfluidic devices, vol 56. Elsevier Science, Amsterdam, pp 267–287
44. Zhu L, Zhang Q, Feng H, Ang S, Chau FS, Liu WT (2004) Filter-based microfluidic device as a platform for immunofluorescent assay of microbial cells. R Soc Chem 4:337–341
45. Wiggins S, Ottino JM (2004) Foundations of chaotic mixing. Philos Trans R Soc Lond A 362: 937–970
46. Air RB (2007) Digital microfluidics: is a true lab-on-a-chip possiblez. Microfluid Nanofluid 3: 245–281
47. Song H, Chen DL, Ismagilov RF (2006) Reactions in droplets in microfluidic channels. Angew Chem Int Ed 45:7336–7356
48. Dolatabadi A, Mohseni K, Arzpeyma A (2006) Behaviour of a moving droplet under electrowetting actuation: numerical simulation. Can J Chem Eng 84:17–21
49. Alphonsus HCN, Uddayasankar U, Wheeler AR (2010) Immunoassays in microfluidic systems. Anal Bioanal Chem 397:991–1007
50. Liu Y-J, Yao D-J, Lin H-C, Chang W-Y, Chang H-Y (2008) DNA ligation of ultramicro volume using an EWOD microfluidic system with coplanar electrodes. J Micromech Microeng 18:045017
51. Fair RB (2007) Digital microfluidics: is a true lab-on-a-chip possible? Microfluid Nanofluid 3: 245–281

52. Miyazaki M, Honda T, Yamaguchi H, Briones MPP, Maeda H (2008) Enzymatic processing in microfluidic reactors. Biotechnol Genet Eng 25:405–428
53. Lee J, Soper SA, Murray KK (2009) Microfluidics with MALDI analysis for proteomics – a review. Anal Chim Acta 649:180–190
54. Figeys D, Gygi SP, McKinnon G, Aebersold R (1998) An integrated microfluidics-tandem mass spectrometry system for automated protein analysis. Anal Chem 70:3738–3734
55. Yuh P-H, Yang C-L, Chang Y-W (2008) BioRoute: a network-flow-based routing algorithm for the synthesis of digital microfluidic biochips. IEEE Trans Comput Aided Design Integr Circ Syst 27(11):1928–1941
56. Guan A, Hamilton P, Wang Y et al (2017) Medical devices on chips. Nat Biomed Eng 1:0045. https://doi.org/10.1038/s41551-017-0045
57. Lee J, Lilly GD, Doty RC, Podsiadlo P, Kotov NA (2009) In vitro toxicity testing of nanoparticles in 3D cell culture. Small 5:1213–1221
58. Marx U et al (2012) 'Human-on-a-chip' developments: a translational cutting-edge alternative to systemic safety assessment and efficiency evaluation of substances in laboratory animals and man? Altern Lab Anim 40:235–257
59. Kang G, Lee JH, Lee CS, Nam Y (2009) Agarose microwell based neuronal micro-circuit arrays on microelectrode arrays for high throughput drug testing. Lab Chip 9:3236–3242. https://doi.org/10.1039/b910738j
60. Wu MH, Huang SB, Lee GB (2010) Microfluidic cell culture systems for drug research. Lab Chip 10:939–956. https://doi.org/10.1039/b921695b
61. Lal R, Arnsdorf MF (2010) Multidimensional atomic force microscopy for drug discovery: a versatile tool for defi ning targets, designing therapeutics and monitoring their efficacy. Life Sci 86:545–562. https://doi.org/10.1016/j.lfs.2009.02.030
62. Terstappen GC, Schlupen C, Raggiaschi R, Gaviraghi G (2007) Target deconvolution strategies in drug discovery. Nat Rev Drug Discov 6:891–903. https://doi.org/10.1038/nrd2410
63. Imming P, Sinning C, Meyer A (2006) Drugs, their targets and the nature and number of drug targets. Nat Rev Drug Discov 5:821–834. https://doi.org/10.1038/nrd2132
64. Dunlop J, Bowlby M, Peri R, Vasilyev D, Arias R (2008) High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. Nat Rev Drug Discov 7: 358–368. https://doi.org/10.1038/nrd2552
65. Suzuki H, Tabata K, Kato-Yamada Y, Noji H, Takeuchi S (2004) Planar lipid bilayer reconstitution with a micro-fluidic system. Lab Chip 4:502–505. https://doi.org/10.1039/b405967k
66. Mayer M, Kriebel JK, Tosteson MT, Whitesides GM (2003) Microfabricated tefl on membranes for low-noise recordings of ion channels in planar lipid bilayers. Biophys J 85: 2684–2689
67. Goldberg MD, Lo RC, Abele S, Macka M, Gomez FA (2009) Development of microfluidic chips for heterogeneous receptor-ligand interaction studies. Anal Chem 81:5095–5098. https://doi.org/10.1021/ac9006649
68. Kang L, Chung BG, Langer R, Khademhosseini A (2008) Microfluidics for drug discovery and development: from target selection to product lifecycle management. Drug Discov Today 13:1–13. https://doi.org/10.1016/j.drudis.2007.10.003
69. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1994) Molecular biology of the cell. Garland Publishing, New York
70. Jendretzki A, Wittland J, Wilk S, Straede A, Heinisch JJ (2011) How do I begin? Sensing extracellular stress to maintain yeast cell wall integrity. Eur J Cell Biol 90:740–744
71. Kultz D, Chakravarty D (2001) Maintenance of genomic integrity in mammalian kidney cells exposed to hyperosmotic stress. Comp Biochem Physiol A Mol Integr Physiol 130:421–428
72. Pipe CJ, McKinley GH (2009) Microfluidicrheometry. Mech Res Commun 36:110–120
73. Lawrence BJ, Madihally SV (2008) Cell colonization in degradable 3D porous matrices. Cell Adhes Migr 2:9–16

74. Shin M, Matsuda K, Ishii O, Terai H, Kaazempur-Mofrad M, Borenstein J, Detmar M, Vacanti JP (2004) Endothelialized networks with a vascular geometry in microfabricated poly (dimethyl siloxane). Biomed Microdevices 6:269–278
75. Fidkowski C, Kaazempur-Mofrad MR, Borenstein J, Vacanti JP, Langer R, Wang Y (2005) Endothelialized microvasculature based on a biodegradable elastomer. Tissue Eng 11:302–309
76. Galan I, Deleon JA, Diaz L, Hong JS, Khalek N, Munoz-Fernandez MA, Santolaya-Forgas J (2007) Effect of a bone marrow microenvironment on the ex-vivo expansion of umbilical cord blood progenitor cells. Int J Lab Hematol 29:58–63
77. Engler AJ, Sen S, Sweeny HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. Cell 126:677–689
78. Bruzewicz DA, McGuigan AP, Whitesides GM (2008) Fabrication of a modular tissue construct in a microfluidic chip. Lab Chip 8:663–671
79. Hong S, Hsu H-J, Kaunas R, Kameoka J (2012) Collagen microsphere production on a chip. Lab Chip 12:3277–3280
80. Sugiura S, Oda T, Izumida Y, Aoyagi Y, Satake M, Ochiai A, Ohkohchi N, Nakajima M (2005) Size control of calcium alginate beads containing living cells using micro-nozzle array. Biomaterials 26:3327–3331
81. Tan WH, Takeuchi S (2007) Monodisperse alginate hydrogel microbeads for cell encapsulation. Adv Mater 19:2696–2701
82. Kumachev A, Greener J, Tumarkin E, Eiser E, Zandstra PW, Kumacheva E (2011) High-throughput generation of hydrogel microbeads with varying elasticity for cell encapsulation. Biomaterials 32:1477–1483
83. Tumarkin E, Tzadu L, Csaszar E, Seo M, Zhang H, Lee A, Peerani R, Purpura K, Zandstra PW, Kumacheva E (2012) High-throughput combinatorial cell co-culture using microfluidics. Integr Biol 3:653–662
84. Castel D, Pitaval A, Debily MA, Gidrol X (2006) Cell microarrays in drug discovery. Drug Discovery Today 11:616–622
85. Skelley AM, Kirak O, Suh H, Jaenisch R, Voldman J (2009) Microfluidic control of cell pairing and fusion. Nature Methods 6:147–152
86. Wood DK, Weingeist DM, Bhatia SN, Engelward BP (2010) Single cell trapping and DNA damage analysis using microwell arrays. Proc Natal Acad Sci U S A 107:10008–10013
87. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE (1997) Geometric control of cell life and death. Science 276:1425–1428
88. Jordan P, Leach J, Padgett M, Blackburn P, Isaacs N, Goksor M, Hanstorp D, Wright A, Girkin J, Cooper J (2005) Creating permanent 3D arrangements of isolated cells using holographic optical tweezers. Lab Chip 5:1224–1228
89. Voldman J, Gray ML, Toner M, Schmidt MA (2002) A microfabrication based dynamic array cytometer. Anal Chem 74:3984–3990
90. Ino K, Okochi M, Konishi N, Nakatochi M, Imai R, Shikida M, Ito A, Honda H (2008) Cell culture arrays using magnetic force-based cell patterning for dynamic single cell analysis. Lab Chip 8:134–142
91. Haake A, Neild A, Kim D-H, Ihm J-E, Sun Y, Dual J, Ju B-K (2005) Manipulation of cells using an ultrasonic pressure field. Ultrasound Med Biol 31:857–864
92. Carlo DD, Wu LY, Lee LP (2006) Dynamic single cell culture array. Lab Chip 6:1445–1449
93. Rettig JR, Folch A (2005) Large-scale single-cell trapping and imaging using microwell arrays. Anal Chem 77:5628–5634
94. Tan W-H, Takeuchi S (2007) A trap-and-release integrated microfluidic system for dynamic microarray applications. Proc Natl Acad Sci U S A 104:1146–1151
95. Carlborg CF, Haraldsson T, Stemme G, Wijngaart WVD (2007) Reliable batch manufacturing of miniaturized vertical vias in soft polymer replica molding. Proceedings of International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), Paris, France, October 7–11, 2007

96. Murphy D (2001) Fundamentals of light microscopy and digital imaging. Wiley-Liss, New York
97. Liu X, Sun Y (2009) Automated mouse embryo injection moves toward practical use. Proceedings of the 2009 IEEE International Conference on Roboticsand Automation. Kobe, Japan, May 12–17, 2009
98. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse-embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676
99. Mason C, Dunnill P (2008) A brief definition of regenerative medicine. Regen Med 3:1–5
100. Burt RK, Testori A, Craig R, Cohen B, Suffi TR, Barr W (2008) Hematopoietic stem cell transplantation for autoimmune diseases: What have we learned? J Autoimmun 30:116–120
101. Sykes M, Nikolic B (2005) Treatment of severe autoimmune disease by stem cell transplantation. Nature 435:620–627
102. Phinney DG, Prockop DJ (2007) Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair – current views. Stem Cells 25: 2896–2902
103. El-Ali J, Sorger PK, Jensen KF (2006) Cells on chips. Nature 442:403–411
104. Meyvantsson I, Beebe DJ (2008) Cell culture models in microfluidic systems. Annu Rev Anal Chem 1:423–449
105. Lanza RP, Hayes JL, Chick WL (1996) Encapsulated cell technology. Nat Biotechnol 14: 1107–1111
106. Wan J (2012) Microfluidic-based synthesis of hydrogel particles for cell microencapsulation and cell-based drug delivery. Polymers 4:1084–1108
107. Song H, Chen DL, Ismagilov RF (2006) Reactions in droplets in microflulidic channels. Angew Chem Int Ed 45:73
108. Martinez AW, Phillips ST, Butte MJ, Whitesides GM (2007) Patterned paper as a platform for inexpensive, low-volume, portable bioassays. Angew Chem Int Ed 46:1318–1320
109. Hawkes R, Niday E, Gordon J (1982) A dot-immunobinding assay for monoclonal and other antibodies. Anal Biochem 119:142–147
110. Cheng CM, Martinez AW, Gong J, Mace CR, Phillips ST, Carrilho E, Mirica KA, Whitesides GM (2010) Paper-based ELISA. Angew Chem Int Ed 49:4771–4774
111. Martinez AW, Phillips ST, Carrilho E, Thomas SW 3rd, Sindi H, Whitesides GM (2008a) Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. Anal Chem 80:3699–3707
112. Lu Y, Shi W, Jiang L, Qin J, Lin B (2009a) Rapid prototyping of paper-based microfluidics with wax for low-cost, portable bioassay. Electrophoresis 30:1497–1500
113. Lu Y, Shi W, Qin J, Lin B (2009b) Fabrication and characterization of paper based microfluidics prepared in nitrocellulose membrane by wax printing. Anal Chem 82:329–335
114. Fenton EM, Mascarenas MR, Lopez GP, Sibbett SS (2008) Multiplex lateral-flow test strips fabricated by two-dimensional shaping. ACS Appl Mater Interfaces 1:124–129
115. Li A, Wang Y, Deng L, Zhao X, Yan Q, Cai Y, Lin J, Bai Y, Liu S, Zhang Y (2013) Use of nitrocellulose membranes as a scaffold in cell culture. Cytotechnology 65(1):71–81
116. Cretich M, Sedini V, Damin F, Pelliccia M, Sola L, Chiari M (2010) Coating of nitrocellulose for colorimetric DNA microarrays. Anal Biochem 397:84–88
117. Dungchai W, Chailapakul O, Henry CS (2010) Use of multiple colorimetric indicators for paper-based microfluidic devices. Anal Chim Acta 674:227–233
118. Dungchai W, Chailapakul O, Henry CS (2009) Electrochemical detection for paper-based microfluidics. Anal Chem 81:5821–5826
119. Apilux A, Dungchai W, Siangproh W, Praphairaksit N, Henry CS, Chailapakul O (2010) Lab-on-paper with dual electrochemical/colorimetric detection for simultaneous determination of gold and iron. Anal Chem 82:1727–1732
120. Yu J, Ge L, Huang J, Wang S, Ge S (2011a) Microfluidic paper-based chemiluminescence biosensor for simultaneous determination of glucose and uric acid. Lab Chip 11:1286–1291

121. Delaney JL, Hogan CF, Tian J, Shen W (2011) Electrogenerated chemiluminescence detection in paper-based microfluidic sensors. Anal Chem 83:1300–1306
122. Ge L, Yan J, Song X, Yan M, Ge S, Yu J (2012) Three-dimensional paper-based electrochemiluminescence immunodevice for multiplexed measurement of biomarkers and point-of-care testing. Biomaterials 33:1024–1031
123. Yan J, Ge L, Song X, Yan M, Ge S, Yu J (2012) Paper-based electrochemiluminescent 3D immunodevice for lab-on-paper, specific, and sensitive point of- care testing. Chemistry 18: 4938–4945
124. Abe K, Suzuki K, Citterio D (2008) Inkjet-printed microfluidic multianalytechemical sensing paper. Anal Chem 80:6928–6934
125. Wang L, Chen W, Xu D, Shim BS, Zhu Y, Sun F, Liu L, Peng C, Jin Z, Xu C (2009) Simple, rapid, sensitive, and versatile SWNT − paper sensor for environmental toxin detection competitive with ELISA. Nano Lett 9:4147–4152
126. Lankelma J, Nie Z, Carrilho E, Whitesides GM (2012) Paper-based analytical device for electrochemical flow-injection analysis of glucose in urine. Anal Chem 84:4147–4152
127. Free MJ (2004) Achieving appropriate design and widespread use of health care technologies in the developing world. Overcoming obstacles that impede the adaptation and diffusion of priority technologies for primary health care. Int J Gynecol Obstet 85:S3–S13
128. Malkin RA (2007) Design of health care technologies for the developing world. Annu Rev Biomed Eng 9:567–587
129. Singer PA, Taylor AD, Daar AS, Upshur REG, Singh JA, Lavery JV (2007) Grand challenges in global health: The ethical, social and cultural program. PLoS Med 4:1440–1444
130. Mackay IM (2004) Real-time PCR in the microbiology laboratory. Clin Microbiol Infect 10: 190–212
131. Foudeh AM, Didar TF, Veres T, Tabrizian M (2012) Microfluidic designs and techniques using lab-on-a-chip devices for pathogen detection for point-of-care diagnostics. Lab Chip 12: 3249–3266
132. Yang S, Rothman RE (2004) PCR-based diagnostics for infectious diseases: Uses, limitations, and future applications in acute-care settings. Lancet Infect Dis 4:337–348
133. Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, Shepherd A, Wilson P, Dahl CA, Buchsbaum S, Moeller G, Hay Burgess DC (2006) Requirements for high impact diagnostics in the developing world. Nature 444(Suppl 1):73–79
134. Gervais L, de Rooij N, Delamarche E (2011) Microfluidic chips for point- of care immunodiagnostics. Adv Mater 23:H151–H176
135. Song LS, Zhang Y, Wang WJ, Ma LY, Liu Y, Hao YL, Shao YM, Zhang W, Jiang XY (2012) Microfluidic assay without blocking for rapid HIV screening and confirmation. Biomed Microdevices 14:631–640
136. Kelly RT, Woolley AT (2005) Microfluidic systems for integrated, high-throughput DNA analysis. Anal Chem 77:96a–102a

Lab-on-a-Chip for Functional Testing for Precision Medicine

27

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Abstract

Microfluidic lab-on-chip technology is a blooming and promising field for functional testing for precision medicine. The current approach of microfluidic technology with multidisciplinary fields enables the development of controlled microenvironments that mimic organ-level physiology. The device is an amalgam of small microchannels for fluid flow, an imaging system, and a computer system for detection and analysis of experimental data. The energetic efforts put into for the discovery of integrated lab-on-chip devices made the performance of biochemical assays, cytotoxic assays, disease detection, and drug discoveries much easier. The use of these devices enables rapid monitoring of health parameters and biomarkers of diseases like cancer and neurodegenerative disorders, which might prevent the occurrence and worsening of the same. This innovative approach poses more advantages than traditional techniques including rapidness, sensitivity, disposability, portability, low cost, and easiness to manufacture. Numerous device-friendly materials and fabrication techniques are available currently. This chapter focuses on latest trends in organ-on-chip, multi-organ-on-chip, and lab-on-chip and their application in precision medicine.

Keywords

Precision medicine · Microfluidics · Organ-on-chip · Multi-organ-on-chip · Lab-on-chip

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27.1 Introduction

In the twenty-first century, people give more attention to disease prevention to ensure a healthy lifestyle. Quality of life mainly depends on good health. Continuous monitoring of health parameters is an integral approach to prevent lifestyle disorders. Precision medicine is a treatment technique that utilizes the information of a person's genes or proteins and lifestyle to diagnose, identify, and treat a particular disease condition. It helps collect specific information about a disorder, analyze and plan a treatment schedule for continuous monitoring of drug acceptance to evaluate the disease prognosis. This approach can reduce the severe side effects caused by certain drugs in the early stages and could replace them with other substitutes. Precision medicine incorporates tools like imaging, molecular diagnostics, and analytics. So the development of miniaturized devices with low cost, rapid detection, and easiness to use is highly appreciated.

The introduction of nanotechnology has had a revolutionizing impact on many fields of science and made human life much easier. The term 'Lab-on-Chip' was coined by Moser et al. [1] for a miniaturized device that works under the principle of microfluidic technology, which fits an operational laboratory onto a tiny chip [2]. Collection, preservation, and transporting of specimens are hazardous and precarious tasks that risks contamination, and false results are likely to occur. Therefore delay in getting results is also a significant concern that hinders quick treatment. Lab-on-Chip is a miniaturized microfluidic platform that allows fluid flow through micro-channels for rapid, sensitive, and highly reliable results in in vitro diagnostics, drug discovery, and several applications in the healthcare system. They act as a tenet for multiple laboratory functions and consist of components like fluidics electronics, optics, and sensing structures. The scope of lab-on-chip devices extends from the determination of water and air quality, biological analysis of human fluids, detection of ions, forensic and agricultural domains [3]. Such lab-on-chip devices act as a platform to conduct biological tests from single-cell analysis to multi-organ levels. Moreover, it reduces in vivo experiments and animal sacrifices [4]. Decreased reagent consumption and limited laboratory space make lab-on-chip a unique and noticeable product. Improvisation of this device on demand by changing the operational fluidic units using integration and fabrication technology makes it a diverse and functional platform for performing analytical assays [5]. "Lab on-chip," a perfect example for "Science and Technology," has gained attention globally due to its beneficial role in interdisciplinary research [6] (Fig. 27.1).

A lab-on-a-chip is incorporated with a microchip or a nanochip, a proper imaging system associated with a computer system for detection and analysis of experimental data [7]. The materials used to fabricate the device varies according to its applications and are required for excellent system performance. Materials used range from glass, ceramics, silicon to polymers like polymethyl methacrylate (PMMA) and polydimethylsiloxane (PDMS). Glass is more preferred than ceramics due to its optical transparency. Gerhardt et al. created a multipurpose single high-pressure resistant lab-on-chip device made of glass to perform High-Performance Liquid Chromatography (HPLC) and droplet microfluidics [8]. Wlodarczyk et al.

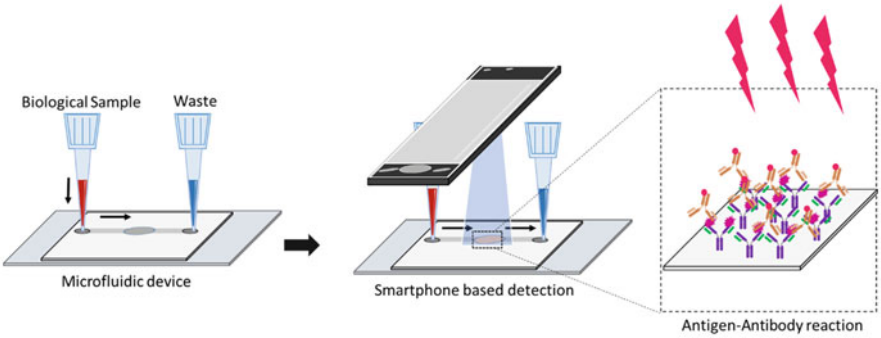

Fig. 27.1 Schematics of a lab-on-a-chip device showing smartphone-based detection of antigen–antibody reaction

introduced new technology to create a glass microfluidic device rather than the conventional time-consuming and multi-step processes using a picosecond pulsed laser [9]. PDMS is commonly used in microfluidic devices in pharmaceutical and diagnostic sectors, but characters like hydrophobicity and the unavailability of a technique to introduce bonding to form PDMS layers restrict it from use. However, in 2020, Oyama et al. applied an electron beam or Gamma Rays to bond the interface of PDMS layers, hinder absorption and adsorption of hydrophobic molecules, and sterilize the internal chambers and microchannels [10]. Moreover, the application of nanotechnology together with innovative fabrication technology for the development of lab on chip devices can enhance the performance of the devices [11]. Another widely used material is PMMA over PDMS; PMMA is an amorphous thermoplastic with good optical transparency, mechanical properties, and solvent compatibility, permitting surface modification and small-scale chip production. Hydrogels and papers are recent alternatives for microfluidic devices [12].

27.2 Latest Trends on Organ-on-Chip for Precision Medicine

Organ-on-chip devices are advanced technology with models of organs and tissues to track metabolic diseases and the effect of drugs on organs. They provide a platform for in-depth research with high content screening and real-time sensing. Blood-Brain Barrier (BBB), a metabolic or immunological barrier, acts as a gatekeeper that restricts the entry of waste products like neurotoxins from the blood to the brain and vice versa. It consists of differentiated brain endothelial cells to maintain brain homeostasis. The cells like astrocytes, microglia, neurons, pericytes, and the BBB together make up the Neurovascular Unit (NVU). BBB- and NVU-on-chip provide new insights for studying schizophrenia, neurodegenerative diseases, and interactions of various drugs, cytokines, antibodies, and metabolites on BBB [3] (Fig. 27.2).

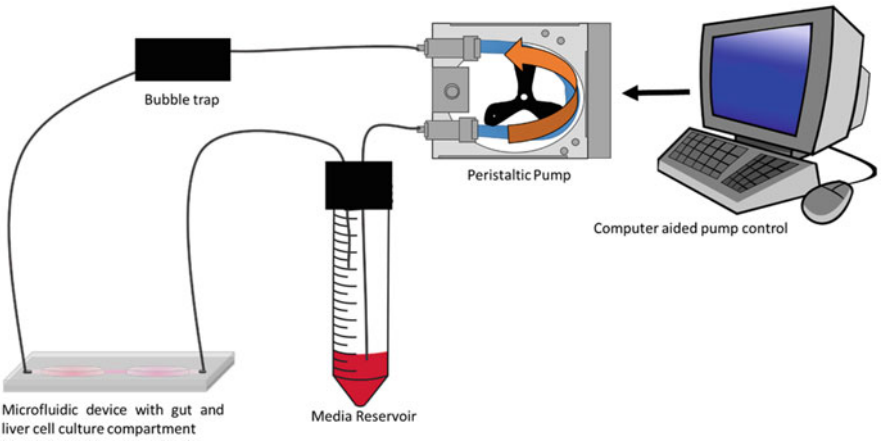

Fig. 27.2 Pictorial representation of experimental setup for metastasis-on-a-chip

Another advancement in Organ-on-chip is the development of tumor-on-chip, an integrated platform of microfluidic technology, 3-dimensional cell cultures, and tissue engineering. It acts as a potential tenet to screen anticancer drugs and treatments for cancer as it provides a microenvironment with all biophysical and biochemical tumor factors, which in vivo animal models and in vitro cell models are unable to impart [13]. Skardal et al. developed a Metastasis-on-chip (MOC) device to study drug screening and tumor progression modelling. The device possesses two gut and liver constructs chambers fabricated with hyaluronic acid-based hydrogel and connected by circulating fluid flow [14]. Incorporating immune cells into tumor-on-chip permits studying the interaction of tumor cells and immune cells [15]. Ayuso et al. used time-lapse microscopy to study the migration of activated natural killer cells to penetrate glioblastoma by tumor-on-chip method [16].

27.2.1 Gut on-Chip (GOC)

Gut on-chip is another remarkable micro-physiological system to monitor intestinal functionalities with real-time sensing methods. Gut microbiota influences the functioning of the human body in many ways, from absorption, transport, and metabolisms of drugs and nutrients [17]. Gut flora influences immunity, and microbiome dysbiosis is sometimes a cause of life-threatening diseases like cancer. In 2018, Workman et al. and team developed intestine-on-chip, integrating human intestinal organoids by extracting epithelial cells from induced pluripotent stem cells. This organoid technology included all intestinal epithelial subtypes, like enterocytes, goblet cells, Paneth cells, and enteroendocrine cells [18].

27.2.2 Islet-on-Chip (IOC)

Islet-on-chip (IOC) devices are a perfect example for organ-on-a-chip device to sense insulin secretion by localized surface plasmon resonance (LSPR), developed by Ortega et al. and team. This novel device is constructed with mouse pancreatic islets attached to the cellulose-based scaffold [19].

27.3 Trends on Multi-Organ-on-Chip for Precision Medicine

The complete potential studies are only possible on a multi-organ chip to improve preclinical testing on precision medicine. MOC allows organ–organ interaction, drug development studies, and multi-functioning organ systems [20]. Chip design and tissue maturation determine the functionality and scope of multi-organ-on-chip. μOrgano is a multi-organ-on-chip or human-on-chip designed by Loskill et al. that allows the development of individual cell cultures and later the interconnection of these cells to form a perfect multi-organ-on-chip device [21]. Oleaga et al. devised a functional in vitro multi-organ-on-chip, consisting of neurons, muscles, heart, and liver cells for toxicity screening. The device was viable for 14 days and tested the toxicity of five drugs [22].

Ong et al. developed a modular microfluidic platform that enabled control of modules of different organs using self-aligning magnetic interconnections and demonstrated the interaction of multi-organs to start liver-mediated activation of nutraceuticals and efficiency of drugs for cancer and atherosclerosis [23]. "Physiome-on-a-chip", devised by Edington et al., is a 7-way interconnected Microphysiological System for pharmacological and quantitative biological studies. This device enables multiple MPS to flow configurations operated by pneumatically driven pumps for controlled distribution of drugs and molecular exchange between MPS [24]. Another unique approach was by Trapecar et al., who developed a MOC with human gut-liver axis and adaptive immune cells to tackle ulcerative colitis (UC) ex vivo. Microbiome derives Short Chain Fatty Acids (SCFA)-enhanced metabolic processes like lipogenesis and glycolysis and production of ketone bodies that ultimately reduce gut immune activation. But surprisingly, SCFA's reprogrammed metabolic processes, by activating CD4+ T cells leading to T cell-mediated inflammation, resulted in hepatic injury and gut-barrier disruption [25].

27.4 Latest Trends on Lab-on-Chip for Precision Medicine

The establishment and remodelling of new materials and fabrication technologies make lab-on-chip devices more pliable and robust. Over the past 5 years, paper-on-chip devices were introduced to improve the sample pretreatment process and detection. This method enhances point-of-care testing, as it doesn't require the need of professionals for sample collection, disposability, portability, cheapness,

and being a simple diagnostic device [26]. Urine and blood are the preferred samples for analysis.

Paper-on-chip acts as the perfect platform for specimen collection by surface treatment like nitrification or application of surfactants [27], fast technical analysis (FTA) [28], or making wearable devices followed by separation of the analyte by dielectrophoresis, capillary force, or by using filter paper [26]. Fabrication of lab-on-paper devices is either by physical or chemical methods [29]. Paper-on-chip devices have been used recently for determining the concentration of glucose [30], serum bilirubin [31], creatinine [32], cardiac troponin [33], triglycerides, and cholesterol [34].

Optofluidic sensors, a collaborated technology of photonics and microfluidics, are the best method for biological and chemical analyses to detect incalculable sample volumes. This integrated platform uses either of the four methods for analyte detection: fluorescence detection, optical trapping and manipulation, RI detection, and surface-enhanced Raman spectroscopy (SERS). In addition, the optical device possesses traditional technologies to perform chromatography and electrophoresis, making it a more advanced tool for biological and chemical analysis [35]. Choi et al. developed an optofluidic biosensor that detects trace amounts of protein aggregates like amyloid substances, embarking an early detection of neurodegenerative diseases like Alzheimer's and Parkinson's [36]. Another loss-based optofluidic miniaturization is an optical trap for particle analysis like *Escherichia coli* [37].

Incorporating magnetic nanoparticles onto lab-on-chip technology ensures fluid manipulation [38] and enhanced cellular separation [39]. Magnetic nanomaterials can act as liquid mixers, carriers, and labels for bioseparation [38]. Xiong et al. created a biochip integrated with magnetic nanomaterials. This multi-specimen analyzer detects bacterial species and potential cancer biomarkers from 1 μL of the specimen within 8 min using Raman enhanced scattering for signal transduction [40]. The combination of nanotechnology and microfluidics to create a next-generation nanomaterial-based microfluidic system has gained attention over the past few years due to its high sensitivity, precision, and uncountable applications in the biomedical sector [41]. Bacterial research greatly benefits from the alliance of nanotechnology and microfluidics, ranging from capturing bacterial cells to its detection [42]. Passive methods like nanopores [43], bendable nanowires [44], channels, nanotextured surfaces, and active methods like electrical and optical fields are used for capturing bacteria using nanotechnology [42]. Nanostructures enhance the surface area for the attachment of bacteria, followed by lysis of the cell by mechanical methods like nano spikes [45].

An alternative method for rapid detection of cancer biomarkers from liquid biopsy specimens is devised by Shi et al. without any damage and a label-free technique by using electrokinetic technologies onto lab-on-chip [46]. Liquid crystals or mesogens are a state between solid and liquid. With microfluidics, liquid crystal (LC) technology is an emerging tool in diagnostics, environmental, and food safety monitoring with real-time detection, low cost, and label-free lab-on-chip devices. Smartphone devices are sufficient for the quantitative determination of different analytes [47]. Choi et al. used liquid crystal microdroplets to detect HepG2 cells

(Human Liver hepatocellular carcinoma) by tracking ligand-receptor interactions [48]. 2-methacryloyloxyethyl phosphorylcholine, a type of phospholipid nanomaterial, is a promising material to be used on lab-on-chip devices for early diagnosis of COVID-19; it possesses resistance to small nonspecific compounds [49].

Saliva is the best specimen to detect the physiological and pathological state of the body since innumerable substances like DNA, RNA, hormones, minerals, enzymes, mucins, and microorganisms can quickly diffuse into them. A novel strategy to detect biomarkers for various diseases can be done by developing a saliva lab-on-chip [50]. Saliva LOC's showed their potential in the detection of several bacteria like *Helicobacter pylori* [51], *Bacillus cereus* [52]; viruses like HIV (Human Immunodeficiency Virus) [53], Zika virus [54], SARS-CoV-2 [55]; Cytokines [56], and oral cancer [57]. Another innovative strategy to sense disease is using immobilized split aptamers in lab-on-chip-based photo-sensors [58]. Short single-stranded RNA or DNA, which can bind non-nucleic acid-like small molecules and proteins substrate molecules with high specificity and affinity, are called aptamers [59]. In 2015, Zhang et al. invented a whole cell-based microfluidic device for the selective capture of live *E. coli* cells [60]. Microfluidic single-cell analysis impersonates deeply into transcriptomics, genomics, proteomics, etc. Tackling intracellular compounds like RNA, DNA, proteins, and amino acids give insights into topics of biological relevance [61]. Some technologies, like Dielectrophoresis (DEP), Droplet, and Microstructure-based single-cell trapping methods, are used in advanced impedance biosensors for single-cell analysis [62]. The first attempt to detect sickle cells by flow cytometry using electrical impedance signals was devised by Liu et al. [63].

Exploiting the lab-on-Chip method for *radiopharmaceutical synthesis* is inevitable because of its role as a diagnostic tool in the various fields of Cardiology, Neurology, Oncology, and inflammation. Positron Emission Tomography (PET) includes assimilating short-lived radioisotopes that emit positron like ^{11}C and ^{18}F and biologically relevant samples. Integration of synthesis device with a purification system reduces the product transfers and minimizes material loss. This also reduces the handling of radioactivity at a particular time [64].

27.5 Applications of Lab-on-Chip for Precision Medicine (Fig. 27.3)

27.5.1 Detection of Disease Markers

Recent studies focus on the detection of biomarkers. Biomarkers indicate whether a disease already exists or there is a chance for the occurrence of a disease. Y. Zhang et al. developed 10–100 times highly sensitive lab-on-chip photonic biosensors for rapid and early diagnosis of cancer. This biosensor, made of silicon or silica, detects disease-associated multiple miRNAs in real-time even from specimens like urine and blood without amplifying or labelling [65]. Advancement in lab-on-chip

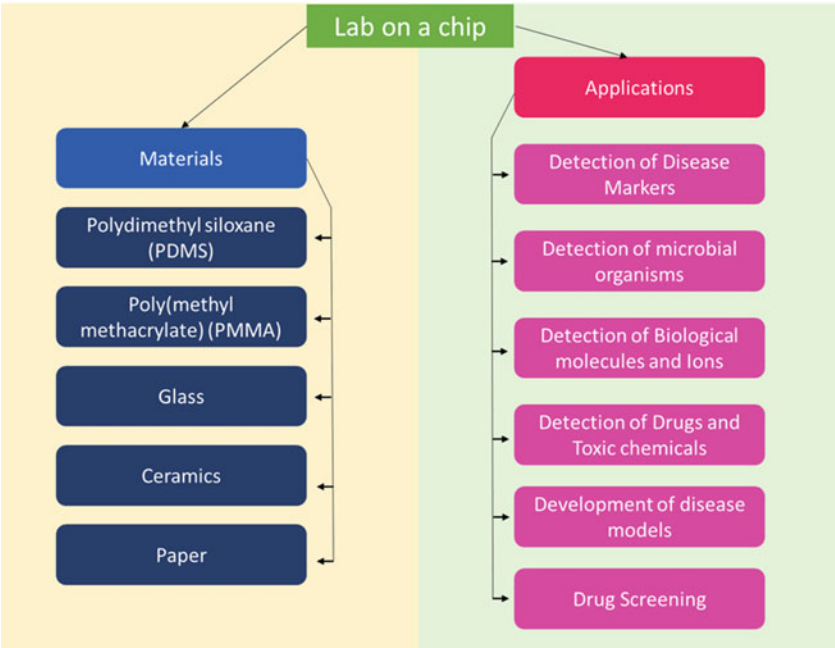

Fig. 27.3 Flow chart depicting various materials used for the fabrication of lab-on-chip devices and their different applications in the biomedical field

technology contributes much to point-of-care (POC) analytical diagnostics. Dai et al. introduced a single microfluidic chip that detects four biomarkers of colorectal cancer using chemiluminescence from human plasma within 20 min [66]. Metastatic Breast Cancers are deleterious when undetected and treated. Alexandrou et al. devised a technique to detect ESR1 mutations by an ion-sensitive field-effect transistor (ISFET) using the lab-on-chip platform, ensuring metastatic monitoring [67]. Mandal et al. introduced microfluidic technology to detect biomarkers of cancer CA-125 using carbon nanotube-based biosensors from micro-volume of biological samples [68]. Lab-on-chip devices can also determine oral cancer from saliva specimens [69].

Glial-fibrillary-acidic protein is a potential biomarker of several diseases, like traumatic brain injuries (TBIs), multiple sclerosis (MS), neuromyelitis optic (NMO), glioblastoma multiforme (GBM), and intracerebral hemorrhage (ICH). In 2021, Agostini et al. developed an ultra-high-frequency surface-acoustic-wave lab-on-chip to detect glial-fibrillary-acidic-protein (GFAP) from serum matrix using gold-coated quartz-crystal-microbalance sensors [70]. Microfluidic technology and lab on-chip platforms have been used recently to study the behavior and assembly of "prion-like" proteins (amyloid proteins) [71]. Arosio et al. characterized the size and interactions of macromolecules-like proteins under native conditions using a hydro-dynamic focusing system to determine the size and quantitative immunoassay to find

the interactions [72]. Some molecular processes can propagate trans-synaptically in neurodegenerative diseases like Alzheimer's and Parkinson's. Deleglise et al. tackled the mechanism of beta-amyloid aggregates on the reconstructed neuronal network using microfluidic technology [73].

27.5.2 Detection of Microbial Organisms

Pathogenic microorganisms are catastrophic, and the need to rapidly detect such organisms is obligatory. Tsougeni et al. demonstrate a close-packed and highly integrated lab-on-chip device for detecting foodborne pathogens like *Salmonella typhimurium*, *Bacillus cereus*, *Listeria monocytogenes*, and *Escherichia coli* from milk. The chip acts as a tenet for label-free detection of the bacteria with Surface Acoustic Wave (SAW) biosensor [74]. Lab-on-chip provides a platform for synthesizing and breaking down substrates into products using different bacteria and enzymes. In 2006, Lee et al. developed a micro-bioreactor to perform fermentation and study the interaction of eight Escherichia coli strains under diverse environmental conditions with strict control on pH and dissolved oxygen [75]. In 2013, Petralia and coworkers manifested a compact, multitasking silicon lab-on-a-chip "In check system" to prepare the sample, PCR amplification, and detect nucleic acids by microarray method directly from biological specimens like blood and cultured cells. Both quantification and application of DNA were possible with the device, ensuring high efficiency and less time [76].

The global healthcare system needs highly reliable, cheap, and rapid devices to detect diseases, as the impact created by COVID-19 is tragic and enigmatic. Hence, optical-on-chip detection of the disease is necessary to stop the outbreak [77]. Viral diagnostics mainly focus on three biomarkers like viral genetic material either DNA or RNA, antibodies in the serum, and viral coat proteins on the envelope [78]. Rapid detection of viruses is possible with graphene-based lab-on-chip devices [2]. In 2020, Zhang et al. devised a fast and facile lab-on-chip device to detect COVID-19 by electrically probing the binding domain of the receptor of the spike protein using Gr-FET (Graphene field-effect transistor) [79]. Hashemi et al. designed a nanosystem based on graphene and gold to detect the ultra-sensitive viral glycoprotein of SARS-CoV-2 [80]. Lab-on-chip devices have already proven their ability in the detection of other viruses like HIV [81], Norovirus [82], Dengue virus [83], Ebola virus [84], and Hepatitis C viruses [85].

27.6 Detection of Biological Molecules and Ions

Devising a single automated device for sample preparation, amplification, and detection for early molecular diagnostics will be a promising method [86]. Monitoring biological molecules and ions can detect the onset of diseases and prevent them from occurring. The blood glucose level of a diabetic patient is much higher than 70–110 mg. Garcia et al. reported the quantitative detection of glucose along with

serum albumin, nitrite, uric acid, etc., on a paper-based-chip manufactured by handheld stamping fabrication technology [87]. V. Srinivasan et al. and team made a completely integrated lab-on-chip device for clinical diagnostics of physiological fluids like saliva, plasma, serum, and urine. The device used electrowetting to move physiological specimens as microdroplets and possess a sample injecting system, mixing area, and detection sites. A glucose assay was performed to convey the proof-of-concept on lab-on-a-chip [88].

Chronic Kidney disease (CKD) can be traced by determining the creatinine concentration. Avila et al. introduced a disposable chip to detect creatinine levels from blood using electrophoretic separation and their detection by conductivity [89]. Buzzin et al. determine hemoglobin concentration from blood using a compact optoelectronic chip. An amorphous silicon photo-sensor is used in the system [90]. Bhat et al. and their team developed the first real-time optofluidic lab-on-chip device for fluoride detection. The device was constructed with PDMS microchannels and UV-visible optical probe for detection [91]. Other ions that microfluidic techniques can detect are lead [92], Iron [93], Copper [94], and Zinc [95]. Phenylketonuria is a hereditary metabolic disease that is to be diagnosed early. Lab-on-paper devices are already developed by Robinson et al. A reaction zone was prepared coated with phenylalanine dehydrogenase enzyme, which underwent a colored enzymatic reaction and was analyzed by colorimetry [96]. Another disease caused by point mutation is Sickle cell Anemia (SCD) due to the abnormal formation of sickle hemoglobin (HbS). Piety et al. developed a low-cost, highly sensing lab-on-the-paper device to detect HbS antigen from blood samples [97].

27.7 Detection of Drugs and Toxic Chemicals

Lab-on-chip devices were recently used to detect the drugs of abuse in over 18 countries. New Psychoactive substances (NPS) and cocaine were mainly studied. Lab-on-chip techniques were preferred for such studies as they are rapid, sensitive, portable, and cheap detectable systems and accept non-biological and biological specimens in forensic and clinical studies [98]. The preclinical evaluation during drug development consists of several phases from target screening, ADMET (absorption, distribution, metabolism, elimination, and toxicity), clinical trials, drug conceptualization, and optimization of the manufacturing process, which is quite a long process [99]. Xiao et al. introduced a cheap microdroplet chip to detect seven drugs from human serum, using electrochemiluminescent array and paper cover fabrication technology, within 1 min. The drugs detected were chlorphenamine, levosulpiride, metoprolol, matrine, linomycin, sophocarpine, and sophoridine [100].

Conducting cytotoxicity assays on two-dimensional cell line cultures doesn't provide in vivo cellular conditions and no scaffold to aid conventional tissue function. Collaborating microfluidic technology to develop a biochip with miniaturized bioreactors, powerful monitoring devices to count viable cells and detect cellular activities will produce sensitive results on cytotoxicity assays. It

also enables the study of the interaction of different cell types [101]. A High Content Screening (HCS) test was performed to determine the human hepatotoxicity of potential drugs. It is a lab-on-Chip with HepG2 human hepatocytes and provides a platform for incubating cells with various drugs. Drugs were characterized based on their site of action in different compartments of cell-like mitochondrial membrane and cell membrane by changing the permeability, which affects intracellular calcium concentration, etc., by rapid image analysis and automated epifluorescence microscopy [102]. Piccino et al. studied the potential of the drug Sorafenib in treating Hepatocellular carcinoma (HCC) on a lab-on-chip platform. The number of cells used was few; real-time detection and lesser reagents were the advantages of this device [103].

27.8 Other Applications of LOC for Precision Medicine

Lab-on-chip devices are examples of potential blood analyzers. Dimov et al. introduced SIMBAS (Stand-alone self-powered integrated microfluidic blood analysis system), an essential contribution to separating red and white blood cells from 5 μL of blood sample within 10 min and doesn't require an external network for transport [104]. Lee et al. developed a novel microfluidic device with a micro-pump-based micromixer to rapidly detect antimicrobial susceptibility. This device even determined the Minimum Inhibitory concentration of multiple antibiotics using calorimetric techniques and studied the interaction effect of combinations of antibiotics. They reported a combination therapy of various antibiotics like ceftazidime and gentamicin to treat carbapenem-resistant *Escherichia coli* [105].

Exosomes and microvesicles are the two central extracellular vesicles that are released to extracellular space and mediate cell–cell communication by transferring signalling molecules like nucleic acids, lipids, and proteins. Current technologies are insufficient for in-depth detection analysis of exosomes and microvesicles [106]. Lab-on-chip platform enhances sorting, counting, conducting studies on the content, lysis, and detecting nucleic acids in exosomes and microvesicles on a single chip. Liang et al. devised an integrated double filtration lab-on-a-chip to detect exosomes of size 30–200 nm from urine and quantify the extracellular vesicles done by microchip ELISA. An elevated level of extracellular vesicles in urine is a biomarker for bladder cancer [107]. Gupta et al. proposed a theranostic implantable-on-chip platform for the diagnosis of hypertension. The device uses blood pressure as the input and produces electrical signals as output in response, which helps determine the stage of hypertension, thus preventing chronic disorders [108]. A Microfluidic Magnetic Separator (MMS) was developed for phage selection depending on the peptide sequence. The device is a promising automated tool for fast in vitro-directed development of affinity reagents, provided stringent washing conditions of selected peptide sequence [109].

Antibody epitope mapping is a two-stage on-chip screening that uses dielectrophoretic funnelling to separate antibody binding target cells. Polymeric beads capture bacterial cells that exhibit peptides complementary to the antibody

binding region, then amplify bacterial cells binding cells and sequence determination. An integrated chip-based technique for disease profiling to detect serum antibodies will be a prominent approach [110].

27.9 Conclusion

To summarise, microfluidic lab-on-chip technology is the highly evolving and flourishing solution for functional testing for precision medicine. The technology possesses numerous advantages over traditional techniques, including sensitivity, rapidness, reliability, easiness to manufacture, low cost, disposability, and portability, making them a promising tool in point-of-care testing. Innovation of such devices helps prepare rapid detection kits, which are much needed during a pandemic. Numerous materials and advanced fabrication technologies are available to develop improvised lab-on-chip devices. Exploiting such innovative combinations for real-time drug screening, immune and cytotoxicity assays, detection of biomarkers of diseases, nanoparticle synthesis, and can contribute much to diagnostic studies. This review mainly focused on the contributions of lab-on-chip devices in precision medicine.

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References

1. Moser I, Jobst G, Aschauer E, Svasek P, Varahram M, Urban G, Zanin VA, Tjoutrina GY, Zharikova AV, Berezov TT (1995) Miniaturized thin film glutamate and glutamine biosensors. Biosens Bioelectron 10(6–7):527–532
2. Sengupta J, Adhikari A, Hussain CM (2021) Graphene-based analytical lab-on-Chip devices for detection of viruses: a review. Carbon Trends 4:100072
3. Staicu CE, Jipa F, Axente E, Radu M, Radu BM, Sima F (2021) Lab-on-a-chip platforms as tools for drug screening in neuropathologies associated with blood–brain barrier alterations. Biomol Ther 11(6):916
4. Azizipour N, Avazpour R, Rosenzweig DH, Sawan M, Ajji A (2020) Evolution of biochip technology: a review from lab-on-a-chip to organ-on-a-chip. Micromachines 11(6):599
5. Mark D, Haeberle S, Roth G, Von Stetten F, Zengerle R (2010) Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications. Microfluidics Microsyst:305–376
6. Zhang Y (2013) Optimal design of label-free silicon "lab on a chip" biosensors. Prog Nat Sci Mater Int 23(5):481–486
7. Mozaffari S, Ghasemi H, Tchoukov P, Czarnecki J, Nazemifard N (2021) Lab-on-a-Chip Systems in Asphaltene characterization: a review of recent advances. Energy Fuel. https://doi.org/10.1021/acs.energyfuels.1c00717
8. Gerhardt RF, Peretzki AJ, Piendl SK, Belder D (2017) Seamless combination of high-pressure chip-HPLC and droplet microfluidics on an integrated microfluidic glass chip. Anal Chem 89(23):13030–13037

9. Wlodarczyk KL, Hand DP, Maroto-Valer MM (2019) Maskless, rapid manufacturing of glass microfluidic devices using a picosecond pulsed laser. Sci Rep 9(1):1–13
10. Oyama TG, Oyama K, Taguchi M (2020) A simple method for production of hydrophilic, rigid, and sterilized multi-layer 3D integrated polydimethylsiloxane microfluidic chips. Lab Chip 20(13):2354–2363
11. Pumera M (2011) Nanomaterials meet microfluidics. Chem Commun 47(20):5671–5680
12. Niculescu AG, Chircov C, Bîrcă AC, Grumezescu AM (2021) Fabrication and applications of microfluidic devices: a review. Int J Mol Sci 22(4):2011
13. Liu X, Fang J, Huang S, Wu X, Xie X, Wang J, Liu F, Zhang M, Peng Z, Hu N (2021) Tumor-on-a-chip: from bioinspired design to biomedical application. Microsyst Nanoeng 7(1):1–23
14. Skardal A, Devarasetty M, Forsythe S, Atala A, Soker S (2016) A reductionist metastasis-on-a-chip platform for in vitro tumor progression modeling and drug screening. Biotechnol Bioeng 113(9):2020–2032
15. Clapp N, Amour A, Rowan WC, Candarlioglu PL (2021) Organ-on-chip applications in drug discovery: an end user perspective. Biochem Soc Trans 49(4):1881–1890
16. Ayuso JM, Truttschel R, Gong MM, Humayun M, Virumbrales-Munoz M, Vitek R et al (2019) Evaluating natural killer cell cytotoxicity against solid tumors using a microfluidic model. OncoImmunology 8:1553477. https://doi.org/10.1080/2162402X.2018.1553477
17. Signore MA, De Pascali C, Giampetruzzi L, Siciliano PA, Francioso L (2021) Gut-on-Chip microphysiological systems: latest advances in the integration of sensing strategies and adoption of mature detection mechanisms. Sens Biosens Res 33:100443
18. Workman MJ, Gleeson JP, Troisi EJ, Estrada HQ, Kerns SJ, Hinojosa CD, Hamilton GA, Targan SR, Svendsen CN, Barrett RJ (2018) Enhanced utilization of induced pluripotent stem cell–derived human intestinal organoids using microengineered chips. Cell Mol Gastroenterol Hepatol 5(4):669–677
19. Ortega MA, Rodríguez-Comas J, Yavas O, Velasco-Mallorquí F, Balaguer-Trias J, Parra V, Novials A, Servitja JM, Quidant R, Ramón-Azcón J (2021) In situ LSPR sensing of secreted insulin in organ-on-Chip. Biosensors 11(5):138
20. Basak S (2020) Unlocking the future: converging multi-organ-on-a-chip on the current biomedical sciences. Emerg Mater 3:693–709
21. Loskill P, Marcus SG, Mathur A, Reese WM, Healy KE (2015) μOrgano: a Lego®-like plug & play system for modular multi-organ-chips. PLoS One 10(10):e0139587
22. Oleaga C, Bernabini C, Smith AS, Srinivasan B, Jackson M, McLamb W, Platt V, Bridges R, Cai Y, Santhanam N, Berry B, Najjar S, Akanda N, Guo X, Martin C, Ekman G, Esch MB, Langer J, Hickman JJ (2016) Multi-organ toxicity demonstration in a functional human in vitro system composed of four organs. Sci Rep 6(1):1–17
23. Ong LJY, Ching T, Chong LH, Arora S, Li H, Hashimoto M, Das Gupta R, Yuen PK, Toh YC (2019) Self-aligning Tetris-like (TILE) modular microfluidic platform for mimicking multi-organ interactions. Lab Chip 19(13):2178–2191
24. Edington CD, Chen WLK, Geishecker E, Kassis T, Soenksen LR, Bhushan BM, Freake D, Kirschner J, Maass C, Tsamandouras N, Valdez J, Cook CD, Parent T, Snyder S, Yu J, Suter E, Shockley M, Velazquez J, Velazquez JJ, Stockdale L, Papps JP, Lee I, Vann N, Gamboa M, ME LB, Zhong Z, Wang X, Boyer LA, Lauffenburger DA, Carrier RL, Communal C, Tannenbaum SR, Stokes CL, Hughes DJ, Rohatgi G, Trumper DL, Cirit M, Griffith LG (2018) Interconnected microphysiological systems for quantitative biology and pharmacology studies. Sci Rep 8(1):1–18
25. Trapecar M, Communal C, Velazquez J, Maass CA, Huang YJ, Schneider K, Wright CW, Butty V, Eng G, Yilmaz O, Trumper D, Griffith LG (2020) Gut-liver physiomimetics reveal paradoxical modulation of IBD-related inflammation by short-chain fatty acids. Cell Syst 10(3):223–239
26. Tai WC, Chang YC, Chou D, Fu LM (2021) Lab-on-paper devices for diagnosis of human diseases using urine samples—a review. Biosensors 11(8):260

27. Tang RH, Yang H, Choi JR, Gong Y, Feng SS, Pingguan-Murphy B, Huang QS, Shi JL, Xu F (2017) Advances in paper-based sample pretreatment for point-of-care testing. Crit Rev Biotechnol 37(4):411–428
28. Srisiri K, Jaroenwattana R, Panvisavas N, Bandhaya A (2017) Optimization of DNA recovery and analysis of urine samples stored on FTA® card. Forensic Sci Int Genet Suppl Ser 6:e520–e522
29. Lee WC, Ng HY, Hou CY, Lee CT, Fu LM (2021) Recent advances in lab-on-paper diagnostic devices using blood samples. Lab Chip. https://doi.org/10.1039/D0LC01304H
30. Cánovas R, Parrilla M, Blondeau P, Andrade FJ (2017) A novel wireless paper-based potentiometric platform for monitoring glucose in blood. Lab Chip 17(14):2500–2507
31. Pan X, Li L, Lin H, Tan J, Wang H, Liao M, Chen C, Shan B, Chen Y, Li M (2019) A graphene oxide-gold nanostar hybrid based-paper biosensor for label-free SERS detection of serum bilirubin for diagnosis of jaundice. Biosens Bioelectron 145:111713
32. Tseng CC, Yang RJ, Ju WJ, Fu LM (2018) Microfluidic paper-based platform for whole blood creatinine detection. Chem Eng J 348:117–124
33. Li F, Guo L, Hu Y, Li Z, Liu J, He J, Cui H (2020) Multiplexed chemiluminescence determination of three acute myocardial infarction biomarkers based on microfluidic paper-based immunodevice dual amplified by multifunctionalized gold nanoparticles. Talanta 207:120346
34. Hsieh HY, Li LH, Hsu RY, Kao WF, Huang YC, Hsu CC (2017) Quantification of endogenous cholesterol in human serum on paper using direct analysis in real time mass spectrometry. Anal Chem 89(11):6146–6152
35. Fan X, White IM (2011) Optofluidic microsystems for chemical and biological analysis. Nat Photonics 5(10):591–597
36. Choi I, Huh YS, Erickson D (2011) Size-selective concentration and label-free characterization of protein aggregates using a Raman active nanofluidic device. Lab Chip 11(4):632–638
37. Kühn S, Measor P, Lunt EJ, Phillips BS, Deamer DW, Hawkins AR, Schmidt H (2009) Loss-based optical trap for on-chip particle analysis. Lab Chip 9(15):2212–2216
38. Arshavsky-Graham S, Segal E (2020) Lab-on-a-chip devices for point-of-care medical diagnostics. Adv Biochem Eng Biotechnol. https://doi.org/10.1007/10_2020_127
39. Stueber DD, Villanova J, Aponte I, Xiao Z, Colvin VL (2021) Magnetic nanoparticles in biology and medicine: past, present, and future trends. Pharmaceutics 13(7):943
40. Xiong Q, Lim CY, Ren J, Zhou J, Pu K, Chan-Park MB, Mao H, Lam YC, Duan H (2018) Magnetic nanochain integrated microfluidic biochips. Nat Commun 9(1):1–11
41. Rabiee N, Ahmadi S, Fatahi Y, Rabiee M, Bagherzadeh M, Dinarvand R, Bagheri B, Zarrintaj P, Saeb MR, Webster TJ (2020) Nanotechnology-assisted microfluidic systems: from bench to bedside. Nanomedicine 16(3):237–258
42. Li L, Wang C, Nie Y, Yao B, Hu H (2020) Nanofabrication enabled lab-on-a-chip technology for the manipulation and detection of bacteria. TrAC Trends Anal Chem 127:115905
43. Guo P, Hall EW, Schirhagl R, Mukaibo H, Martin CR, Zare RN (2012) Microfluidic capture and release of bacteria in a conical nanopore array. Lab Chip 12(3):558–561
44. Liu L, Chen S, Xue Z, Zhang Z, Qiao X, Nie Z, Han D, Wang J, Wang T (2018) Bacterial capture efficiency in fluid bloodstream improved by bendable nanowires. Nat Commun 9(1):1–9
45. Li L, Tian F, Chang H, Zhang J, Wang C, Rao W, Hu H (2019) Interactions of bacteria with monolithic lateral silicon nanospikes inside a microfluidic channel. Front Chem 7:483
46. Shi L, Esfandiari L (2022) Emerging on-chip electrokinetic based technologies for purification of circulating cancer biomarkers towards liquid biopsy–a review. Electrophoresis. https://doi.org/10.1002/elps.202100234
47. Khoshbin Z, Abnous K, Taghdisi SM, Verdian A (2021) Liquid crystal-based biosensors as lab-on-Chip tools: promising for future on-site detection test kits. TrAC Trends Anal Chem 142:116325

48. Choi Y, Lee K, Gupta KC, Park SY, Kang IK (2015) The role of ligand–receptor interactions in visual detection of HepG2 cells using a liquid crystal microdroplet-based biosensor. J Mater Chem B 3(44):8659–8669
49. Rahimnejad M, Rabiee N, Ahmadi S, Jahangiri S, Sajadi SM, Akhavan O, Saeb MR, Kwon W, Kim M, Hahn SK (2021) Emerging phospholipid Nanobiomaterials for biomedical applications to lab-on-a-Chip, drug delivery, and cellular engineering. ACS Appl Biomater. https://doi.org/10.1021/acsabm.1c00932
50. Mohammadi MH, Mulder S, Khashayar P, Kalbasi A, Azimzadeh M, Aref AR (2021) Saliva lab-on-a-chip biosensors: recent novel ideas and applications in disease detection. Microchem J 168:106506
51. Mannoor MS, Tao H, Clayton JD, Sengupta A, Kaplan DL, Naik RR, Verma N, Omenetto FG, McAlpine MC (2012) Graphene-based wireless bacteria detection on tooth enamel. Nat Commun 3:763
52. Chen Z, Mauk MG, Wang J, Abrams WR, Corstjens PL, Niedbala RS, Malamud D, Bau HH (2007) A microfluidic system for saliva-based detection of infectious diseases. Ann N Y Acad Sci 1098:429–436
53. Chen Z, Zhu H, Malamud D, Barber C, Ongagna YYS, Yasmin R, Modak S, Janal MN, Abrams WR, Montagna RA (2016) A rapid, self-confirming assay for HIV: simultaneous detection of anti-HIV antibodies and viral RNA. J AIDS Clin Res 7:540
54. Sabalza M, Yasmin R, Barber CA, Castro T, Malamud D, Kim BJ, Zhu H, Montagna RA, Abrams WR (2018) Detection of Zika virus using reverse-transcription LAMP coupled with reverse dot blot analysis in saliva. PLoS One 13:e0192398
55. Linnes JC, Sritong N, Colbert A, Ejendal KFK (2020) Micro-rapid autonomous analytical device for sars-cov-2 detection, MicroTAS 2020 - 24th International Conference on Miniaturized Systems for Chemistry and Life Sciences, pp 1222–1223
56. Hao Z, Pan Y, Shao W, Lin Q, Zhao X (2019) Graphene-based fully integrated portable nanosensing system for on-line detection of cytokine biomarkers in saliva. Biosens Bioelectron 134:16–23
57. Lin Y-H, Wu C-C, Peng Y-S, Wu C-W, Chang Y-T, Chang K-P (2018) Detection of anti-p53 autoantibodies in saliva using microfluidic chips for the rapid screening of oral cancer. RSC Adv 8:15513–15521
58. Tombelli S, Minunni M, Mascini M (2005) Analytical applications of aptamers. Biosens Bioelectron 20(12):2424–2434
59. Nandimandalam M, Costantini F, Lovecchio N, Iannascoli L, Nascetti A, de Cesare G, Caputo D, Manetti C (2021) Split aptamers immobilized on polymer brushes integrated in a lab-on-Chip system based on an Array of amorphous silicon Photosensors: a novel sensor assay. Materials 14(23):7210
60. Zhang C, Lv X, Han X, Man Y, Saeed Y, Qing H, Deng Y (2015) Whole-cell based aptamer selection for selective capture of microorganisms using microfluidic devices. Anal Methods 7(15):6339–6345
61. Chao TC, Ros A (2008) Microfluidic single-cell analysis of intracellular compounds. J R Soc Interface 5(suppl_2):S139–S150
62. Kim S, Song H, Ahn H, Kim T, Jung J, Cho SK, Shin D-M, Choi J-R, Hwang Y-H, Kim K (2021) A review of advanced impedance biosensors with microfluidic chips for single-cell analysis. Biosensors 11(11):412
63. Liu J, Qiang Y, Alvarez O, Du E (2018) Electrical impedance microflow cytometry with oxygen control for detection of sickle cells. Sensors Actuators B Chem 255:2392–2398
64. Elizarov AM (2009) Microreactors for radiopharmaceutical synthesis. Lab Chip 9(10): 1326–1333
65. Najeeb N, Zhang Y, Mellor C, Benson T (2015) Photonic biosensor chip for early-stage cancer diagnosis. In: 2015 17th international conference on transparent optical networks (ICTON). IEEE, New York, pp 1–4

66. Dai B, Yin C, Wu J, Li W, Zheng L, Lin F, Han X, Fu Y, Zhang D, Zhuang S (2021) A flux-adaptable pump-free microfluidics-based self-contained platform for multiplex cancer biomarker detection. Lab Chip 21(1):143–153
67. Alexandrou G, Moser N, Mantikas KT, Rodriguez-Manzano J, Ali S, Coombes RC, Shaw J, Georgiou P, Toumazou C, Kalofonou M (2021) Detection of multiple breast cancer ESR1 mutations on an ISFET based lab-on-chip platform. IEEE Trans Biomed Circuits Syst 15(3): 380–389
68. Mandal D, Nunna BB, Zhuang S, Rakshit S, Lee ES (2018) Carbon nanotubes based biosensor for detection of cancer antigens (CA-125) under shear flow condition. Nanostruct Nanoobjects 15:180–185
69. Madhura MG, Rao RS, Patil S, Fageeh HN, Alhazmi A, Awan KH (2020) Advanced diagnostic aids for oral cancer. Dis Mon 66(12):101034
70. Agostini M, Amato F, Vieri ML, Greco G, Tonazzini I, Baroncelli L, Caleo M, Vannini E, Santi M, Signore G, Cecchini M (2021) Glial-fibrillary-acidic-protein (GFAP) biomarker detection in serum-matrix: functionalization strategies and detection by an ultra-high-frequency surface-acoustic-wave (UHF-SAW) lab-on-chip. Biosens Bioelectron 172:112774
71. Jose A, Ferrer I (2020) Potential of microfluidics and lab-on-Chip platforms to improve understanding of "prion-like" protein assembly and behavior. Front Bioeng Biotechnol 8: 570692
72. Arosio P, Müller T, Rajah L, Yates EV, Aprile FA, Zhang Y, Cohen SI, White DA, Herling TW, De Genst EJ, Linse S, Vendruscolo M, Dobson CM, Knowles TP (2016) Microfluidic diffusion analysis of the sizes and interactions of proteins under native solution conditions. ACS Nano 10(1):333–341
73. Deleglise B, Magnifico S, Duplus E, Vaur P, Soubeyre V, Belle M, Vignes M, Viovy JL, Jacotot E, Peyrin JM, Brugg B (2014) β-Amyloid induces a dying-back process and remote trans-synaptic alterations in a microfluidic-based reconstructed neuronal network. Acta Neuropathol Commun 2(1):1–9
74. Tsougeni K, Kaprou G, Loukas CM, Papadakis G, Hamiot A, Eck M, Rabusg D, Kokkorisa G, Chatzandroulisa S, Papadopoulosa V, Dupuye B, Jobstf G, Gizelicd E, Tserepiab A, Gogolides E (2020) Lab-on-Chip platform and protocol for rapid foodborne pathogen detection comprising on-chip cell capture, lysis, DNA amplification and surface-acoustic-wave detection. Sensors Actuators B Chem 320:128345
75. Lee HL, Boccazzi P, Ram RJ, Sinskey AJ (2006) Microbioreactor arrays with integrated mixers and fluid injectors for high-throughput experimentation with pH and dissolved oxygen control. Lab Chip 6(9):1229–1235
76. Petralia S, Verardo R, Klaric E, Cavallaro S, Alessi E, Schneider C (2013) In-check system: a highly integrated silicon lab-on-Chip for sample preparation, PCR amplification and microarray detection of nucleic acids directly from biological samples. Sensors Actuators B Chem 187:99–105
77. Shi Y, Li Z, Liu PY, Nguyen BTT, Wu W, Zhao Q, Chin LK, Wei M, Yap PH, Zhou X, Zhao H, Yu D, Tsai DP, Liu AQ (2021) On-Chip Optical detection of viruses: a review. Adv Photonics Res 2(4):2000150
78. Tymm C, Zhou J, Tadimety A, Burklund A, Zhang JX (2020) Scalable COVID-19 detection enabled by lab-on-chip biosensors. Cell Mol Bioeng 13(4):313–329
79. Zhang X, Qi Q, Jing Q, Ao S, Zhang Z, Ding M, Wu M, Liu K, Wang W, Ling Y, Zhang Z, Fu W (2020) Electrical probing of COVID-19 spike protein receptor binding domain via a graphene field-effect transistor. arXiv preprint arXiv:2003.12529
80. Hashemi SA, Behbahan NGG, Bahrani S, Mousavi SM, Gholami A, Ramakrishna S, Firoozsani M, Moghadami M, Lankarani KB, Omidifar N (2021) Ultra-sensitive viral glycoprotein detection NanoSystem toward accurate tracing SARS-CoV-2 in biological/non-biological media. Biosens Bioelectron 171:112731

81. Gong Q, Yang H, Dong Y, Zhang W (2015) A sensitive impedimetric DNA biosensor for the determination of the HIV gene based on electrochemically reduced graphene oxide. Anal Methods 7(6):2554–2562
82. Ahmed SR, Takemeura K, Li TC, Kitamoto N, Tanaka T, Suzuki T, Park EY (2017) Size-controlled preparation of peroxidase-like graphene-gold nanoparticle hybrids for the visible detection of norovirus-like particles. Biosens Bioelectron 87:558–565
83. Jin SA, Marinero EE, Stanciu LAS, Poudyal S, Kuhn RJ (2017) Impedimetriczika and dengue biosensor based on functionalized graphene oxide wrapped silica particles. In APS March meeting abstracts; vol 2017, pp E5-010
84. Fu MQ, Wang XC, Dou WT, Chen GR, James TD, Zhou DM, He XP (2020) Supramolecular fluorogenic peptide sensor array based on graphene oxide for the differential sensing of ebola virus. Chem Commun 56(43):5735–5738
85. Kim, S., Ryoo, S. R., Na, H. K., Kim, Y. K., Choi, B. S., Lee, Y., .Kim DE Min, D. H. (2013). Deoxyribozyme-loaded nano-graphene oxide for simultaneous sensing and silencing of the hepatitis C virus gene in liver cells. Chem Commun, 49(74), 8241–8243
86. Cunha ML, da Silva SS, Stracke MC, Zanette DL, Aoki MN, Blanes L (2021) Sample preparation for lab-on-a-chip systems in molecular diagnosis: a review. Anal Chem. https://doi.org/10.1021/acs.analchem.1c04460
87. de Tarso Garcia P, Cardoso TMG, Garcia CD, Carrilho E, Coltro WKT (2014) A handheld stamping process to fabricate microfluidic paper-based analytical devices with chemically modified surface for clinical assays. RSC Adv 4(71):37637–37644
88. Srinivasan V, Pamula VK, Fair RB (2004) An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids. Lab Chip 4(4):310–315
89. Wu J, Dong M, Rigatto C, Liu Y, Lin F (2018) Lab-on-chip technology for chronic disease diagnosis. NPJ Digit Med 1(1):1–11
90. Buzzin A, Asquini R, Caputo D, de Cesare G (2021) On-glass integrated SU-8 waveguide and amorphous silicon photosensor for on-Chip detection of biomolecules: feasibility study on hemoglobin sensing. Sensors 21(2):415
91. Bhat MP, Kurkuri M, Losic D, Kigga M, Altalhi T (2021) New optofluidic based lab-on-a-chip device for the real-time fluoride analysis. Anal Chim Acta 1159:338439
92. Khoshbin Z, Housaindokht MR, Izadyar M, Verdian A, Bozorgmehr MR (2019) A simple paper-based aptasensor for ultrasensitive detection of lead (II) ion. Anal Chim Acta 1071:70–77
93. Li JJ, Wang XF, Huo DQ, Hou CJ, Fa HB, Yang M, Zhang L (2017) Colorimetric measurement of Fe3+ using a functional paper-based sensor based on catalytic oxidation of gold nanoparticles. Sensors Actuators B Chem 242:1265–1271
94. Vishaka HV, Saxena M, Chandan HR, Ojha AA, Balakrishna RG (2019) Paper based field deployable sensor for naked eye monitoring of copper (II) ions; elucidation of binding mechanism by DFT studies. Spectrochim Acta A Mol Biomol Spectrosc 223:117291
95. Sutariya PG, Soni H, Gandhi SA, Pandya A (2019) Luminescent behavior of pyrene-allied calix[4]arene for the highly pH-selective recognition and determination of Zn 2+, Hg 2+ and I− via the CHEF-PET mechanism: computational experiment and paper-based device. New J Chem 43(25):9855–9864
96. Robinson R, Wong L, Monnat RJ, Fu E (2016) Development of a whole blood paper-based device for phenylalanine detection in the context of PKU therapy monitoring. Micromachines 7(2):28
97. Piety NZ, Yang X, Kanter J, Vignes SM, George A, Shevkoplyas SS (2016) Validation of a low-cost paper-based screening test for sickle cell anemia. PLoS One 11(1):e0144901
98. McNeill L, Megson D, Linton PE, Norrey J, Bradley L, Sutcliffe OB, Shaw KJ (2021) Lab-on-a-Chip approaches for the detection of controlled drugs, including new psychoactive substances: a systematic review. Forensic Chem 26:100370
99. Wen Y, Yang ST (2008) The future of microfluidic assays in drug development. Expert Opin Drug Discovery 3(10):1237–1253

100. Xiao Y, Xu L, Li P, Tang XC, Qi LW (2017) A simple microdroplet chip consisting of silica nanochannel-assisted electrode and paper cover for highly sensitive electrochemiluminescent detection of drugs in human serum. Anal Chim Acta 983:96–102
101. Yang ST, Zhang X, Wen Y (2008) Microbioreactors for high-throughput cytotoxicity assays. Curr Opin Drug Discov Devel 11(1):111–127
102. O'Brien PJ, Irwin W, Diaz D, Howard-Cofield E, Krejsa CM, Slaughter MR, Gao B, Kaludercic N, Angeline A, Bernardi P, Brain P, Hougham C (2006) High concordance of drug-induced human hepatotoxicity with in vitro cytotoxicity measured in a novel cell-based model using high content screening. Arch Toxicol 80(9):580–604
103. Piccinno E, Monteduro AG, Dituri F, Rizzato S, Giannelli G, Maruccio G (2021) Validation of a lab-on-Chip assay for measuring Sorafenib effectiveness on HCC cell proliferation. Int J Mol Sci 22(23):13090
104. Dimov IK, Basabe-Desmonts L, Garcia-Cordero JL, Ross BM, Ricco AJ, Lee LP (2011) Stand-alone self-powered integrated microfluidic blood analysis system (SIMBAS). Lab Chip 11(5):845–850
105. Lee WB, Chien CC, You HL, Kuo FC, Lee MS, Lee GB (2021) Rapid antimicrobial susceptibility tests on an integrated microfluidic device for precision medicine of antibiotics. Biosens Bioelectron 176:112890
106. Chiriacò MS, Bianco M, Nigro A, Primiceri E, Ferrara F, Romano A, Quattrini A, Furlan R, Arima V, Maruccio G (2018) Lab-on-chip for exosomes and microvesicles detection and characterization. Sensors 18(10):3175
107. Liang LG, Kong MQ, Zhou S, Sheng YF, Wang P, Yu T, Inci F, Kuo WP, Li LJ, Demirci U, Wang S (2017) An integrated double-filtration microfluidic device for isolation, enrichment and quantification of urinary extracellular vesicles for detection of bladder cancer. Sci Rep 7(1):1–10
108. Gupta S, Kakkar V, Ahmed S, Khanday FA, Sharma S, Singh S, Yoon B (2021) Modeling of on-Chip biosensor for the in vivo diagnosis of hypertension in wireless body area networks. IEEE Access 9:95072–95082
109. Liu Y, Adams JD, Turner K, Cochran FV, Gambhir SS, Soh HT (2009) Controlling the selection stringency of phage display using a microfluidic device. Lab Chip 9(8):1033–1036
110. Bessette PH, Hu X, Soh HT, Daugherty PS (2007) Microfluidic library screening for mapping antibody epitopes. Anal Chem 79(5):2174–2178

Tumour-on-a-Chip: Perfusion Systems to Model the Extracellular Breast Tumour Microenvironment—From Tumour Progression to Metastasis Formation

28

Annalisa Tirella

Abstract

The integration of three-dimensional (3D) tumour models in perfusion systems has provided new tools to study cancer and metastasis. Such systems are designed to have a high degree of control over biophysical properties of the extracellular tumour microenvironment (TME), and cellular composition. Advancement in 3D models, tissue engineering, biomaterials and microfluidics has allowed an extra level of control over spatial and temporal properties of the TME, and multiplexed in vitro models helped in enhancing knowledge on tumour development, progression and formation of distal metastasis. Advanced 3D tumour in vitro models are nowadays used not only to understand tumour development but also to provide tools for testing therapies and improve preclinical drug development. Breast cancer is one of the most diagnosed types of cancer, with high incidence of recurrent metastasis to bone. Many in vitro models are designed to mimic breast cancer and metastasis to bone and used in early metastatic detection, drug screening and therapy intervention. In this chapter, physical properties of the breast TME are discussed with specific reference to perfusion and microfluidic systems. Advantages of perfusion systems to control flow rates, shear stresses and transmission of mechanical forces, nutrient and oxygen delivery are presented. Ultimately, breast cancer cells' extravasation and formation of distal metastasis to bone-like recipient are discussed with critical perspective to current knowledge-gaps.

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28.1 Introduction

Breast and prostate cancers are the most prevalent killers globally [1–3]. Between these, 80% of patients with advanced breast or prostate cancer develop incurable bone metastases [4–6]. Metastasis and related dynamic processes are mainly unknown. Cancer cells can remain in a dormant state at the bone metastatic site for years, and factors triggering relapse remain unknown. Once triggered, bone metastases have devastating effects in patients being associated with pain, fractures, spinal cord compression and life-threatening hypercalcaemia. Furthermore, the interface between tumour and cells resident in the bone that controls bone homeostasis results in changes to bone architecture and biophysical characteristics. The overall impact of cancer cell growth in bone manifests in fracture, debilitating pain and morbidity. Exact mechanisms for cancer cell metastasis to bone is currently unknown despite the advances in molecular characterization of primary tumours [1, 2].

To date, preclinical models of bone metastasis do not enable stringent, controllable modelling of bone microenvironmental and biophysical cues, and in vivo models fail to recapitulate dissemination patterns observed in cancer patients. There remains a pressing need for a greater understanding of the cancer–bone interface and for novel therapeutic approaches that block both cancer growth and pathological bone-remodelling at the metastatic site. For this reason, in vitro models are highly required to study 1) the mechanisms driving targeted metastases, 2) the changes in both the primary tumour and the metastatic niche and 3) to better understand the disease and identify appropriate treatments. The metastatic niche is hypothesized to create a permissive environment for the establishment of metastatic growth that can be influenced by prior cancer treatment and/or patient co-morbidities. Once within the metastatic niche, modification in cancer cell phenotype drives towards treatment resistance such that pharmacological interventions required to ameliorate cancer growth within the bone do not match those for the primary tumour [7].

Metastatic prostate and breast cancer cells share similar but different biomolecular pathways that maintain different osteogenic and osteoclastic balances, resulting in different lesions. Metastatic breast cancer cells secrete parathyroid hormone related proteins to promote bone growth, but they can also promote the secretion of receptor activator of NF-κB-ligand from osteoblasts which bind to osteoclasts to initiate bone breakdown, with the released growth factors further intensifying bone destructive process [8]. In contrast, prostate cancer cells disrupt bone homeostasis in a rather more osteoblastic manner via the release of growth factors such as bone-morphogenetic proteins, transforming growth factor-β and other growth factors and cytokines [9]. In addition, processes degrading bone microarchitecture (e.g. bone mineral density, BMD), typically associated with ageing, worsen with cancer therapies [10, 11]. Most of the studies in the past decades have used two-dimensional (2D) cell cultures, spheroids and 3D in vitro models, as well as mouse models. In the latter, factors such as substrate chemistry, micro- and nano-structures, flow, perfusion and biomechanical conditions are poorly controlled.

Over the past decade, several in vitro models have been developed and used to study metastasis [12–20]. The main advantage of such models for cancer research is that conditions and properties of the microenvironment are well controlled, allowing precise definition of experimental conditions. However, a broad spectrum of in vitro models of the metastatic cascade have been developed: mostly focusing on the simple static migration and invasion assays. The use of perfusion systems has opened the possibility to design more sophisticated experimental conditions and methods to study metastatic onsets, better recapitulating the human in vivo setting. Developed models can be classified in two categories: the first category looks at the intravasation and/or extravasation in a single system, understanding adhesion of a circulating tumour cell to the microvasculature endothelium [13, 21, 22]; the other, instead, connects different tissues (e.g. organ-on-a-chip) to understand the interplay between specific tissues following the "seed and soil" hypothesis and focusing on the role and variations of tumour microenvironment properties (e.g. matrix components, stiffness, acidosis, hypoxia) [23–29].

The tumour microenvironment (TME) possesses specific roles during specific stages of cancer progression and metastasis [18]. In this work, existing perfusion models of breast tumour are reviewed, with focus on properties of the cellular and extracellular TME. Factors influencing breast cancer metastasis are presented and discussed together with the current challenges to model the complex variation within the TME that drive the formation and activation of metastasis. The use of advanced 3D in vitro models offer the possibility to design and control such properties in physiological range of interest and enhance understanding on how changes in bone-pathophysiology associated with therapy and/or patient characteristics impact metastatic colonization and associated bone-remodelling.

28.2 Parameters to Be Designed into 'Tumour-on-a-Chip' to Reconstitute the Physiological Tumour Microenvironment

Tumour in in vitro models are designed to mimic several steps of the metastatic cascade from primary tumour local invasion to extravasation in secondary sites; more recently, models have been developed to better study metastatic onsets in highly controlled microenvironments. In this section, the principal parameters to be considered to design perfusion system to mimic the TME are described.

A significant risk factor for breast cancer incidence and progression is breast density/stiffness. However, how this links to the biological cascade that underpins the ability of breast cancer cells to metastasize and colonize bone is unknown. What we know is that breast cancer develops in complex tissue environments, enabling sustained growth, invasion and metastasis of cancer cells. The extracellular matrix (ECM), cell types and their cross-talk are key to the remodelling of the TME, with increasing evidence that composition and properties of the TME could be used as predictors of clinical prognosis in breast tumours and metastasis. In particular, clinical studies have identified primary tumours that differ in composition of both

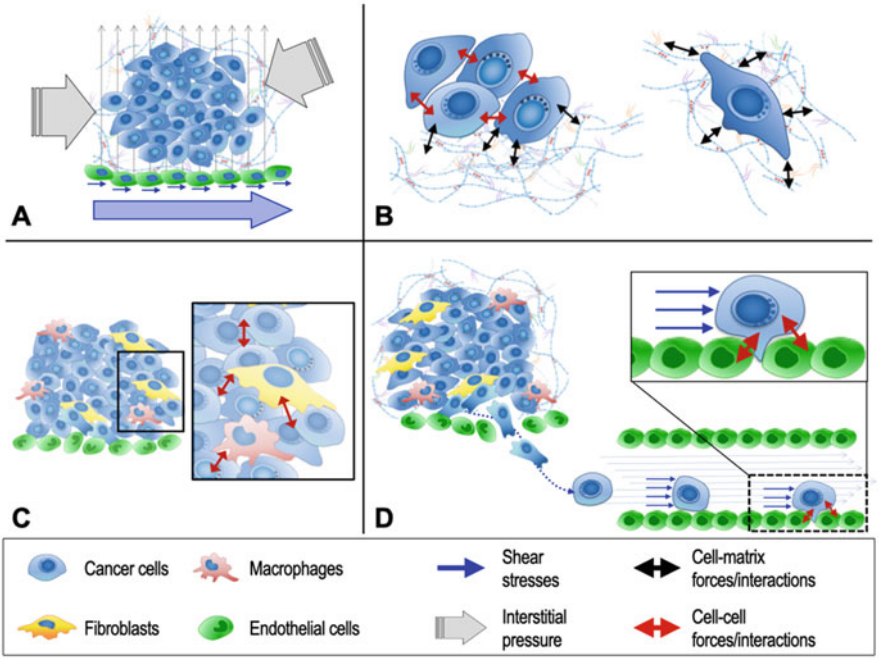

Fig. 28.1 Key parameters to be designed into 'tumour-on-a-chip' to reconstitute the forces and mechanical alterations associated with tumour growth and acting within the tumour microenvironment: (**a**) Tumour-associated variations in interstitial pressure and fluid flow (grey arrows) and shear stresses acting on endothelial cells (blue arrows); (**b**) The extracellular components of the tumour microenvironment consists of several distinct components, including proteins and glycoproteins; during tumour progression, the composition of the extracellular matrix varies, causing an increase of stiffness, and dynamic mechano-transduction. During tumour growth, forces between cancer cells (red arrows, cell-cell interactions) and with the extracellular matrix (black arrows, cell-matrix interactions) progressively increase as the matrix stiffens (left). Invasive/aggressive cancer cells invade the extracellular matrix, exerting forces (black arrows, cell-matrix interactions) in several directions (right). (**c**) Cellular components of the tumour microenvironment (cancer cells, fibroblast, macrophages and endothelial cells): the insert highlights forces (red arrows) between different cell types. (**d**) Escape of cancer cells from the primary tumour site: cancer cells spread locally (within the extracellular components of the tumour microenvironment) and/or distally by travelling through the blood or the lymphatic system to form a new tumour in other regions of the body. The insert highlights the intravasation processes, initial step to form distal metastasis

tumour- and stroma-derived ECM components, which have different metastatic potential. Figure 28.1 summarizes the physical properties of the TME considered to impact on bone metastasis formation: these are urged to be included in advanced in vitro models to better understand tumour progression and metastasis.

28.2.1 Interstitial Fluid Flow, Tumour-Associated Changes in Interstitial Pressure and Compressive Stresses

Fluid flow, interstitial shear forces and indirect biomechanical forces to the cells via the ECM have been shown to play a critical role in mimicking the TME in vitro [30, 31]. Interstitial fluid flow is reported to have typical flow velocities in the range of 0.1–4.0μm/s in tissues, being particularly important in tumorigenesis for the delivery and provision of nutrients and factors; with neoplastic tissue often associated to localized increases in interstitial pressure and pressure gradients at the tumour edge [32]. Perfusion and microfluidic systems have been designed to model tumours in vitro and to have higher degree of control over biomechanical forces typical of extracellular TME, such as interstitial flows and shear stresses (Fig. 28.1a). Complex interactions among multiple cell types can be also modelled [33]. Another advantage of such models is the use of small reagent volumes compared with traditional assays [34]. Moreover, perfusion systems are required to transport nutrients, oxygen and generate spatiotemporal tuneable chemical gradients (e.g. soluble factors) [35].

28.2.2 The Extracellular Matrix and the Key Non-cellular Component of the TME

Breast tissue viscoelasticity is recognized to drive development of breast cancer [36], and increase in mammary tissue rigidity has been quantified during tumour progression from ~1–4 kPa to ~20 kPa [37, 38]. The use of novel biomaterials enables development of 3D in vitro models that mimic the extracellular TME of many tumours. For example, hydrogels have been used to mimic the stiffness of distinct stages of breast cancer progression and 3D bioactive scaffolds to mimic the bone microenvironment of the target metastatic site. Modified polymers are used to design tuneable hydrogels mimicking the dynamic of the TME [16, 39, 40], enabling precise control over viscoelastic properties and to study how changes in extracellular matrix stiffness affect cell phenotypes over time. Cell encapsulation techniques enable facile screening of cancer cell phenotype, viability and proliferation have been reported to study how cancer cells biologically respond to the physical properties of the encapsulating biomaterial [41]. Bone is a tissue stiffer than breast, with known viscoelastic behaviour and stiffness value in the range 5–10 GPa [42]. In recent years, bone scaffold development has focused on the use of bioactive components in polymeric matrices to recapitulate the viscoelastic properties and the bioactivity of the tissue. Poly-?-caprolactone (PCL) matrix, recently, has shown to better support osteoblast growth [43, 44] and enabling precise 3D printing of scaffolds with controlled pore size and porosity.

28.2.3 Cellular Interactions Within the Tumour Microenvironment: Stroma and Vasculature

The tumour stroma is composed of (newly) deposited ECM and multiple cells types; among these, the most important are cancer-associated fibroblasts (CAFs), endothelial cells, pericytes, immune cells and monocytes (simplified sketch in Fig. 28.1c). Stromal cells are known to crosstalk with cancer cells, being key players in regulating secretion of factors and promoting tumour growth [45]. Many fluidic systems have been designed to study interactions with stroma, with main focus on the re-modelling of the extracellular TME. In these, the presence of fluid flow has the main role to provide exchange of nutrients and provision of factors, as well as guarantee cross-talk between stromal and cancer cells [46]. 3D scaffolds are often included to give support to cell adhesion and growth. Langer et al. have modelled different tumour phenotypes in vitro using 3D printed systems [47]. Breast cancer cells (MCF-7, SKBR3, HCC1143 and MDA-MB-231) have been co-cultured with several stromal cells (e.g. adipocytes, fibroblasts, endothelial cells) and used to assess therapeutic efficacy in remodelled 3D systems. Inclusion of 3D co-culture models in perfusion systems allows a finer control over extracellular TME and its remodelling, and will be discussed in the following sections.

Different is the approach used to study the tumour vascular responses [48]. Most of such systems are designed to understand the role of interstitial flow on tumour cells, and more recently refined to include the response of endothelial cells during perfusion and allowing remodelling during tumour and angiogenic progression [49–51]. Gioiella et al. reported on the co-culture of epithelial and cancer cells in a microfluidic chip. The system was designed to monitor changes in the ECM and show the possibility to incorporate endothelial cells in side channels to study the therapeutic effect of drugs on both cellular and ECM compartment [52].

28.2.4 Endothelial Layer and Cancer Cells Circulation

The escape of tumour cells from the primary site, and the following escape into the blood stream is a critical step in cancer metastasis (Fig. 28.1d). Over the past decade, significant progresses have been made in modelling this process in vitro and better understand cell motility in vivo. However, the underlying mechanisms of cancer cells' intravasation and metastatic onsets remain largely unknown. To expand the studies on perfusion of co-culture 3D models of cancer and endothelial cells, 3D co-culture dynamic systems specifically designed to study carcinoma cells invasion and intravasation processes are required [53, 54]. Recently, Nguyen et al. reviewed the microfluidic technologies and models developed to study the vasculature systems discussing the ability of such systems to study mechanobiology and stages of pathologies in highly controlled microenvironments [55]. Chen et al. described an in vitro model to recapitulate and study early stages of metastasis using a microfluidic system [14]. The perfusion system allowed the study of extravasation in different conditions, varying the cellular and extracellular TME, with the potential

to be used for the study of (micro)metastasis formation. Zervantonakis et al. reported a qualitative evaluation of dynamic interactions of tumour-endothelial cells and their migration across the ECM-endothelial channel interface. The study reports a faster migration speed when cells are exposed to TNF-α (2 ng/mL), suggesting that impaired endothelial barrier function facilitates faster tumour cell invasion [20].

28.3 Breast Cancer In Vitro Models: Past, Present and Future

Several tumour-on-a-chip devices have been developed and described in literature, and as previously reported. Advancements in the biomedical and bioengineering field could help to understand breast cancer evolution and consequent therapies [56]. Figure 28.2 summarizes the trend of publications using keywords such as "breast cancer," "microfluidics" and "in vitro models". Over the past decade, an increase in publications in multiple disciplines was produced (Fig. 28.2b), yet there is still the need to develop better models to understand how the disease progresses and forms distal metastases.

28.3.1 Modelling the Primary Site

Tumour-on-a-chip models have the main advantage of monitoring system development and allowing for drug screening; although, their main limitations reside in the use of small volumes and on simplified cell–cell and cell–matrix interactions. Perfusion systems with larger tumour volumes are beneficial to better engineer the TME, allowing inclusion of different cell types arranged in an appropriate architecture, providing cross-talk between multiple types, and most importantly, preserve the cellular phenotype in the presence of well-defined flow characteristics [49]. Following this approach, Marshall et al. reported on a perfusion bioreactor with 3D scaffold to mimic breast cancer in vitro. In this system, the viability of cancer cells and normal breast fibroblasts was monitored over time, showing higher values in perfused models [57]. A perfusion system described by Buchanan et al. was designed to work with a controlled range of physiological flow rates (10–200 mm/s, Poiseuille profile) and shear stresses (1–10 dyn/cm^2), with values measured non-invasively in the perfusion system using microparticle image velocimetry method [49]. The 3D perfusion model allows for dynamic co-culture of tumour and endothelial cells and was used to evaluate changes in endothelial morphologies due to shear stresses variations, as well as study the vasculature integrity and angiogenesis in a tumour model. The study reports that, in the presence of flow, all tumour-expressed proangiogenic genes (MMP9, PDGFB, ANGPT2, VEGFA) were significantly upregulated during co-culture.

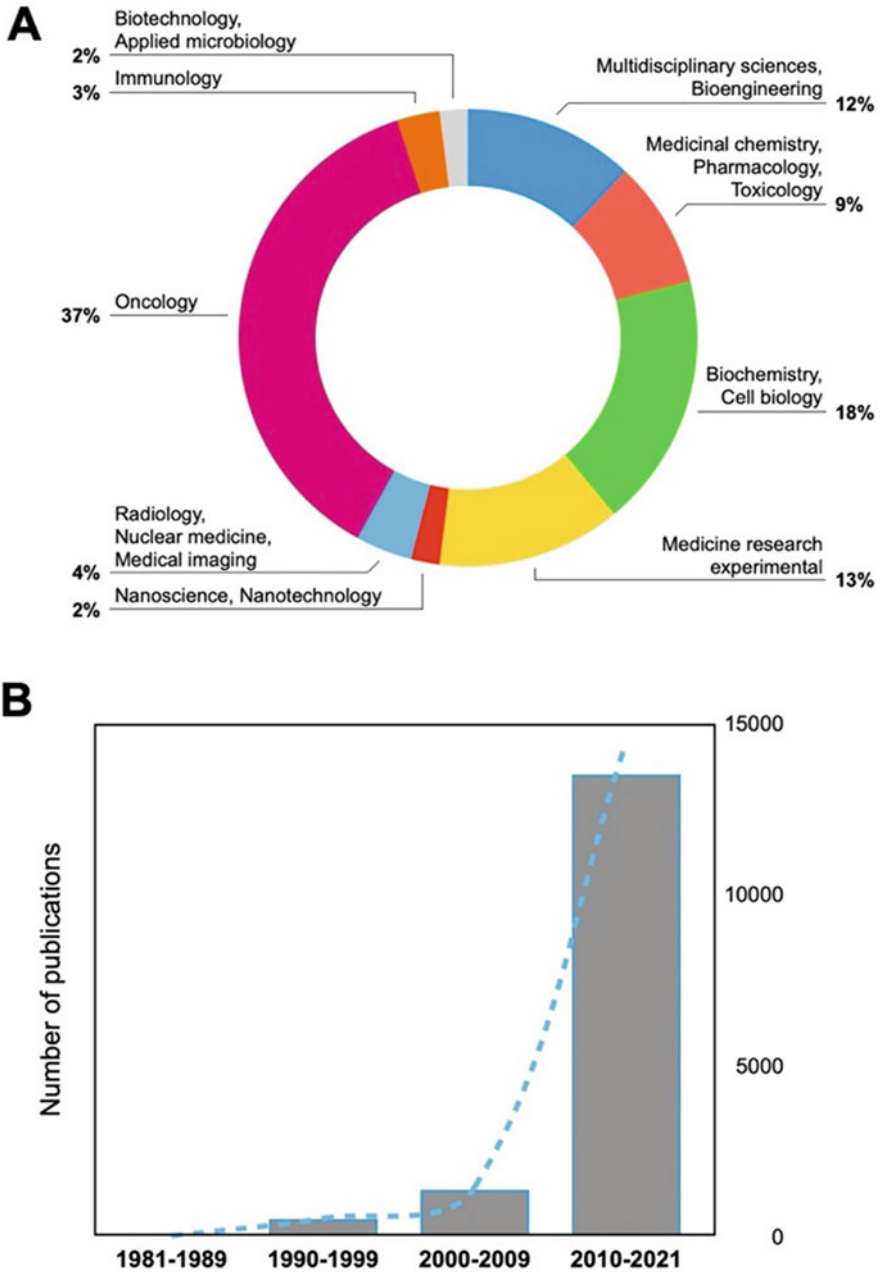

Fig. 28.2 Published papers in Multidisciplinary Sciences between 1981 and 2021 using breast AND tumour OR cancer models AND "in vitro" key words [source web of knowledge]: (**a**) Percentage of publication in different research fields (Oncology 37%; Biochemistry, Cell biology 18%; Medicine Research Experimental 13%; Multidisciplinary Sciences, Bioengineering 12%; Medicinal Chemistry, Pharmacology, Toxicology 9%; Radiology, Nuclear Medicine, Medical Imaging 4%; Immunology 3%; Biotechnology, Applied Microbiology 2%; Nanoscience,

28.3.2 Breast-to-Bone In Vitro Models

To study cell motility and migration, several in vitro models are available and used to investigate the role of cellular and extracellular TME, as well as perfusion and shear stresses. Jeon et al. evaluated the extravasation of breast cancer cells in a human micro-vascularized bone-mimicking microenvironment using a perfusion system [58]. The microfluidic model was designed to control the flow of metastatic breast cancer cells (MDA-MB-231) into a microvascular network, following rolling and adhesion, to an endothelium layer. Further invasion and cellular phenotypes were evaluated, finding that flow-conditioned breast cancer (velocity of 220μm/s, wall shear stress of 0.25 dyn/cm^2) cells have higher motility into the surrounding matrix than unconditioned cells.

Recently Kong et al. designed a microfluidic model to study organ-specific metastasis of circulating tumour cells [15]. Breast cancer cells (MCF-7, MDA-MB-231 and ACC-M cells) were allowed to circulate at a controlled flow rate (750 nL/min), mimicking the dynamic adhesion of circulating tumour cells to endothelium. Adhesion in multiple organs was tested, and results showed significantly higher metastatic potentials to lung, liver and bone marrow compartments. 3D organotypic microfluidic assays to study breast cancer cell extravasation and migration of highly metastatic breast cancer cells in a microfluidic system with the inclusion of a bone-like microenvironment have been reported as tools to expand knowledge of metastasis formation and test efficacy of anti-cancer treatments [12, 58]. Marturano-Kruik et al. designed a perfused bone perivascular niche-on-a-chip to study and evaluate breast tumour progression and drug resistance after colonization of the bone perivascular compartment [59]. Optimized interstitial flow rate of 0.25μm/s was used to perfuse the bone microenvironments, and breast cancer cells were then introduced in the system to mimic metastatic colonisation. However, only few cells colonized the bone microenvironment and only in the absence of fluid flow. Drug studies were performed in the presence of flow to test effective immunotherapy strategies in a physiologically relevant context. As discussed, hydrogels are designed to mimic breast cancer extracellular matrix, allowing encapsulation of breast cancer cell populations in 3D models (Fig. 28.3a). These 3D models allow study of changes occurring in both the cellular (e.g. expression of cell surface markers associated with disease progression CD24, CD44) [41] and extracellular matrix (e.g. remodelling, stiffening). Migration of breast-cancer towards bone-like scaffolds (example in Fig. 28.3b) can be tested assembling both models in static systems (Fig. 28.3c), with the possibility to include osteoblasts on polymeric bone-scaffolds, including bioactive components and study co-culture systems [44]. Advanced models combining different materials to model tissue-specific properties can be integrated in a perfusion system (Fig. 28.3d) to

Fig. 28.2 (continued) Nanotechnology 2%); (**b**) Number of publications in the period 1981–2021 showing increased publications in the past 10 years

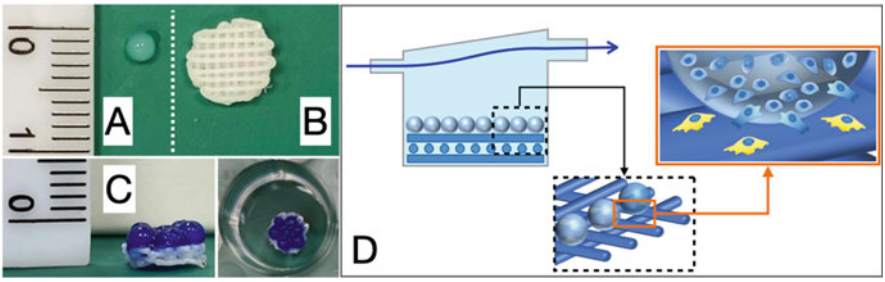

Fig. 28.3 Example of breast-to-bone metastatic model in a perfusion system. The breast-to-bone engineered models use biomaterials-matching properties of target tissues (mechanical, biochemical) and capture physiologically relevant tumour biophysical features (shear stresses, pH, oxygen levels). The main feature of the perfusion system relies in its ability to provide high-nutrient turnover to cells/scaffolds without imposing high-shear stress. The breast-to-bone engineered model will use biomaterials matching properties of target tissues (mechanical, biochemical) and will capture physiologically relevant tumour biophysical features (shear stresses, pH, oxygen levels)

model migration towards distal secondary metastatic site, and to test the metastatic onsets and invasion of breast-to-bone. Such models could help to better understand the effect of bone ECM on cancer cell proliferation, and potentially inform/identify treatments.

28.4 Conclusions and Future Perspectives

The engineering of more realistic in vitro 3D tissue models for the development, testing and screening of cancer therapies/drugs is of upmost importance for the biomedical field. Advanced biomedical materials, additive manufacturing and the integration of 3D scaffolds in perfusion in vitro systems enable the understanding of metastatic onset of many solid cancers. Moreover, the higher degree of control over TME physico-chemical properties enables a high-throughput platform to better study cell–cell and cell–matrix interactions in different extracellular TME. Such models can potentially be translated into scaled-up platforms for drug/therapy testing.

Bone metastasis formation and treatment clearly impose multi-faceted challenges, in which processes occurring via different physical and biological interactions in multi-spatial and temporal scales remain still unknown or not completely deciphered. Current inter-disciplinary research is moving towards the development of unique technologies that allow precise temporal and spatial regulation of biophysical characteristics enabling to address such challenges. The next generation of advanced 3D microfluidic culture in vitro models needs to integrate patient-derived cells in engineered TME, with precise control over multiple biochemical, biophysical and biomechanical cues of specific stages of tumour development. Such technologies will not only allow prediction of metastasis and variation of physiological cues but also will aid understanding of characteristics of the cancer-metastasis

interface and testing of novel therapeutic strategies for better translation to clinical settings.

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References

1. Harbeck N et al (2019) Breast cancer. Nat Rev Dis Primers 5(1):66. https://doi.org/10.1038/s41572-019-0111-2. (In eng)
2. Rebello RJ et al (2021) Prostate cancer. Nat Rev Dis Primers 7(1):9. https://doi.org/10.1038/s41572-020-00243-0. (In eng)
3. Siegel RL, Miller KD, Jemal A (2020) Cancer statistics. CA Cancer J Clin 70(1):7–30. https://doi.org/10.3322/caac.21590. (In eng)
4. D'Oronzo S, Wood S, Brown JE (2021) The use of bisphosphonates to treat skeletal complications in solid tumours. Bone 147:115907. https://doi.org/10.1016/j.bone.2021.115907. (In eng)
5. Thibaudeau L, Quent VM, Holzapfel BM, Taubenberger AV, Straub M, Hutmacher DW (2014) Mimicking breast cancer-induced bone metastasis in vivo: current transplantation models and advanced humanized strategies. Cancer Metastasis Rev 33(2–3):721–735. https://doi.org/10.1007/s10555-014-9499-z. (In eng)
6. Gandaglia G et al (2015) Impact of the site of metastases on survival in patients with metastatic prostate cancer. Eur Urol 68(2):325–334. https://doi.org/10.1016/j.eururo.2014.07.020. (In eng)
7. Gandaglia G et al (2014) Distribution of metastatic sites in patients with prostate cancer: a population-based analysis. Prostate 74(2):210–216. https://doi.org/10.1002/pros.22742. (In eng)
8. Clines GA, Guise TA (2008) Molecular mechanisms and treatment of bone metastasis. Expert Rev Mol Med 10:e7. https://doi.org/10.1017/S1462399408000616. (In eng)
9. Wong SK, Mohamad NV, Giaze TR, Chin KY, Mohamed N, Ima-Nirwana S (2019) Prostate cancer and bone metastases: the underlying mechanisms. Int J Mol Sci 20(10). https://doi.org/10.3390/ijms20102587. (In eng)
10. Miyashita H, Cruz C, Malamud S (2020) Risk factors for skeletal-related events in patients with bone metastasis from breast cancer undergoing treatment with zoledronate. Breast Cancer Res Treat 182(2):381–388. https://doi.org/10.1007/s10549-020-05712-4. (In eng)
11. VanderWalde A, Hurria A (2011) Aging and osteoporosis in breast and prostate cancer. CA Cancer J Clin 61(3):139–156. https://doi.org/10.3322/caac.20103. (In eng)
12. Bersini S et al (2014) A microfluidic 3D in vitro model for specificity of breast cancer metastasis to bone. Biomaterials 35(8):2454–2461. https://doi.org/10.1016/j.biomaterials.2013.11.050. (In eng)
13. Bittner KR, Jiménez JM, Peyton SR (2020) Vascularized biomaterials to study cancer metastasis. Adv Healthc Mater 9(8):e1901459. https://doi.org/10.1002/adhm.201901459. (In eng)
14. Chen MB, Whisler JA, Fröse J, Yu C, Shin Y, Kamm RD (2017) On-chip human microvasculature assay for visualization and quantification of tumor cell extravasation dynamics. Nat Protoc 12(5):865–880. https://doi.org/10.1038/nprot.2017.018. (In eng)
15. Kong J et al (2016) A novel microfluidic model can mimic organ-specific metastasis of circulating tumor cells. Oncotarget 7(48):78421–78432. https://doi.org/10.18632/oncotarget.9382. (In eng)

16. Pradhan S, Slater JH (2019) Tunable hydrogels for controlling phenotypic cancer cell states to model breast cancer dormancy and reactivation. Biomaterials 215:119177. https://doi.org/10.1016/j.biomaterials.2019.04.022. (In eng)
17. Qiao GL, Song LN, Deng ZF, Chen Y, Ma LJ (2018) Prognostic value of CD44v6 expression in breast cancer: a meta-analysis. Onco Targets Ther 11:5451–5457. https://doi.org/10.2147/OTT.S156101. (In eng)
18. Quail DF, Joyce JA (2013) Microenvironmental regulation of tumor progression and metastasis. Nat Med 19(11):1423–1437. https://doi.org/10.1038/nm.3394. (In eng)
19. Wu W, Chen L, Wang Y, Jin J, Xie X, Zhang J (2020) Hyaluronic acid predicts poor prognosis in breast cancer patients: a protocol for systematic review and meta analysis. Medicine (Baltimore) 99(22):e20438. https://doi.org/10.1097/MD.0000000000020438. (In eng)
20. Zervantonakis IK, Hughes-Alford SK, Charest JL, Condeelis JS, Gertler FB, Kamm RD (2012) Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. Proc Natl Acad Sci U S A 109(34):13515–13520. https://doi.org/10.1073/pnas.1210182109. (In eng)
21. Bersini S, Jeon JS, Moretti M, Kamm RD (2014) In vitro models of the metastatic cascade: from local invasion to extravasation. Drug Discov Today 19(6):735–742. https://doi.org/10.1016/j.drudis.2013.12.006. (In eng)
22. Kim S, Lee H, Chung M, Jeon NL (2013) Engineering of functional, perfusable 3D microvascular networks on a chip. Lab Chip 13(8):1489–1500. https://doi.org/10.1039/c3lc41320a. (In eng)
23. Puls TJ, Tan X, Husain M, Whittington CF, Fishel ML, Voytik-Harbin SL (2018) Development of a novel 3D tumor-tissue invasion model for high-throughput, high-content phenotypic drug screening. Sci Rep 8(1):13039. https://doi.org/10.1038/s41598-018-31138-6. (In eng)
24. DelNero P et al (2015) 3D culture broadly regulates tumor cell hypoxia response and angiogenesis via pro-inflammatory pathways. Biomaterials 55:110–118. https://doi.org/10.1016/j.biomaterials.2015.03.035. (In eng)
25. Fane M, Weeraratna AT (2020) How the ageing microenvironment influences tumour progression. Nat Rev Cancer 20(2):89–106. https://doi.org/10.1038/s41568-019-0222-9. (In eng)
26. Taubenberger AV et al (2019) 3D microenvironment stiffness regulates tumor spheroid growth and mechanics via p21 and ROCK. Adv Biosyst 3(9):e1900128. https://doi.org/10.1002/adbi.201900128. (In eng)
27. Cavo M, Fato M, Peñuela L, Beltrame F, Raiteri R, Scaglione S (2016) Microenvironment complexity and matrix stiffness regulate breast cancer cell activity in a 3D in vitro model. Sci Rep 6:35367. https://doi.org/10.1038/srep35367. (In eng)
28. Singh A, Brito I, Lammerding J (2018) Beyond tissue stiffness and bioadhesivity: advanced biomaterials to model tumor microenvironments and drug resistance. Trends Cancer 4(4):281–291. https://doi.org/10.1016/j.trecan.2018.01.008. (In eng)
29. Xu X, Farach-Carson MC, Jia X (2014) Three-dimensional in vitro tumor models for cancer research and drug evaluation. Biotechnol Adv 32(7):1256–1268. https://doi.org/10.1016/j.biotechadv.2014.07.009. (In eng)
30. Karami D, Richbourg N, Sikavitsas V (2019) Dynamic in vitro models for tumor tissue engineering. Cancer Lett 449:178–185. https://doi.org/10.1016/j.canlet.2019.01.043. (In eng)
31. Shieh AC (2011) Biomechanical forces shape the tumor microenvironment. Ann Biomed Eng 39(5):1379–1389. https://doi.org/10.1007/s10439-011-0252-2. (In eng)
32. Wu M, Frieboes HB, McDougall SR, Chaplain MA, Cristini V, Lowengrub J (2013) The effect of interstitial pressure on tumor growth: coupling with the blood and lymphatic vascular systems. J Theor Biol 320:131–151. https://doi.org/10.1016/j.jtbi.2012.11.031. (In eng)
33. Pedersen JA, Lichter S, Swartz MA (2010) Cells in 3D matrices under interstitial flow: effects of extracellular matrix alignment on cell shear stress and drag forces. J Biomech 43(5):900–905. https://doi.org/10.1016/j.jbiomech.2009.11.007. (In eng)

34. Huang Y, Agrawal B, Sun D, Kuo JS, Williams JC (2011) Microfluidics-based devices: New tools for studying cancer and cancer stem cell migration. Biomicrofluidics 5(1):13412. https://doi.org/10.1063/1.3555195. (In eng)
35. Fleury ME, Boardman KC, Swartz MA (2006) Autologous morphogen gradients by subtle interstitial flow and matrix interactions. Biophys J 91(1):113–121. https://doi.org/10.1529/biophysj.105.080192. (In eng)
36. Stowers RS et al (2017) Extracellular matrix stiffening induces a malignant phenotypic transition in breast epithelial cells. Cell Mol Bioeng 10(1):114–123. https://doi.org/10.1007/s12195-016-0468-1. (In eng)
37. Ondeck MG et al (2019) Dynamically stiffened matrix promotes malignant transformation of mammary epithelial cells via collective mechanical signaling. Proc Natl Acad Sci U S A 116(9):3502–3507. https://doi.org/10.1073/pnas.1814204116. (In eng)
38. Wullkopf L et al (2018) Cancer cells' ability to mechanically adjust to extracellular matrix stiffness correlates with their invasive potential. Mol Biol Cell 29(20):2378–2385. https://doi.org/10.1091/mbc.E18-05-0319. (In eng)
39. Narayan OP, Mu X, Hasturk O, Kaplan DL (2021) Dynamically tunable light responsive silk-elastin-like proteins. Acta Biomater 121:214–223. https://doi.org/10.1016/j.actbio.2020.12.018. (In eng)
40. Tirella A, Mattei G, La Marca M, Ahluwalia A, Tirelli N (2020) Functionalized enzyme-responsive biomaterials to model tissue stiffening. Front Bioeng Biotechnol 8:208. https://doi.org/10.3389/fbioe.2020.00208. (In eng)
41. De La Rosa R, Manuel J, Wubetu J, Tirelli N, Tirella A (2018) Colorectal tumor 3D in vitro models: advantages of biofabrication for the recapitulation of early stages of tumour development. Biomed Phys Eng Expr 4(4):045010. https://doi.org/10.1088/2057-1976/aac1c9
42. Wu Z, Ovaert TC, Niebur GL (2012) Viscoelastic properties of human cortical bone tissue depend on gender and elastic modulus. J Orthop Res 30(5):693–699. https://doi.org/10.1002/jor.22001. (In eng)
43. Pierantozzi MD, Scalzone A, Jindal S, Stīpniece L, Šalma-Ancāne K, Dalgarno K, Gentile P, Elena M (2020) 3D printed Sr-containing composite scaffolds: effect of structural design and material formulation towards new strategies for bone tissue engineering. Compos Sci Technol 191:108069. https://doi.org/10.1016/j.compscitech.2020.108069
44. Mancuso E et al (2021) Additively manufactured BaTiO. Mater Sci Eng C Mater Biol Appl 126:112192. https://doi.org/10.1016/j.msec.2021.112192. (In eng)
45. Ramamonjisoa N, Ackerstaff E (2017) Characterization of the tumor microenvironment and tumor-stroma interaction by non-invasive preclinical imaging. Front Oncol 7:3. https://doi.org/10.3389/fonc.2017.00003. (In eng)
46. Polacheck WJ, Charest JL, Kamm RD (2011) Interstitial flow influences direction of tumor cell migration through competing mechanisms. Proc Natl Acad Sci U S A 108(27):11115–11120. https://doi.org/10.1073/pnas.1103581108. (In eng)
47. Langer EM et al (2019) Modeling tumor phenotypes in vitro with three-dimensional bioprinting. Cell Rep 26(3):608–623.e6. https://doi.org/10.1016/j.celrep.2018.12.090. (In eng)
48. Kwak TJ, Lee E (2020) In vitro modeling of solid tumor interactions with perfused blood vessels. Sci Rep 10(1):20142. https://doi.org/10.1038/s41598-020-77180-1. (In eng)
49. Buchanan CF, Voigt EE, Szot CS, Freeman JW, Vlachos PP, Rylander MN (2014) Three-dimensional microfluidic collagen hydrogels for investigating flow-mediated tumor-endothelial signaling and vascular organization. Tissue Eng Part C Methods 20(1):64–75. https://doi.org/10.1089/ten.TEC.2012.0731. (In eng)
50. Stroock AD, Fischbach C (2010) Microfluidic culture models of tumor angiogenesis. Tissue Eng Part A 16(7):2143–2146. https://doi.org/10.1089/ten.TEA.2009.0689. (In eng)
51. Cross VL et al (2010) Dense type I collagen matrices that support cellular remodeling and microfabrication for studies of tumor angiogenesis and vasculogenesis in vitro. Biomaterials 31(33):8596–8607. https://doi.org/10.1016/j.biomaterials.2010.07.072. (In eng)

52. Gioiella F, Urciuolo F, Imparato G, Brancato V, Netti PA (2016) An engineered breast cancer model on a chip to replicate ecm-activation in vitro during tumor progression. Adv Healthc Mater 5(23):3074–3084. https://doi.org/10.1002/adhm.201600772. (In eng)
53. Bhat SM et al (2021) 3D tumor angiogenesis models: recent advances and challenges. J Cancer Res Clin Oncol 147(12):3477–3494. https://doi.org/10.1007/s00432-021-03814-0. (In eng)
54. Pisano M, Triacca V, Barbee KA, Swartz MA (2015) An in vitro model of the tumor-lymphatic microenvironment with simultaneous transendothelial and luminal flows reveals mechanisms of flow enhanced invasion. Integr Biol (Camb) 7(5):525–533. https://doi.org/10.1039/c5ib00085h. (In eng)
55. Nguyen N et al (2021) Microfluidic models of the human circulatory system: versatile platforms for exploring mechanobiology and disease modeling. Biophys Rev 13(5):769–786. https://doi.org/10.1007/s12551-021-00815-8. (In eng)
56. Harbeck N (2020) Breast cancer is a systemic disease optimally treated by a multidisciplinary team. Nat Rev Dis Primers 6(1):30. https://doi.org/10.1038/s41572-020-0167-z. (In eng)
57. Marshall LE, Goliwas KF, Miller LM, Penman AD, Frost AR, Berry JL (2017) Flow-perfusion bioreactor system for engineered breast cancer surrogates to be used in preclinical testing. J Tissue Eng Regen Med 11(4):1242–1250. https://doi.org/10.1002/term.2026. (In eng)
58. Jeon JS et al (2015) Human 3D vascularized organotypic microfluidic assays to study breast cancer cell extravasation. Proc Natl Acad Sci U S A 112(1):214–219. https://doi.org/10.1073/pnas.1417115112. (In eng)
59. Marturano-Kruik A et al (2018) Human bone perivascular niche-on-a-chip for studying metastatic colonization. Proc Natl Acad Sci U S A 115(6):1256–1261. https://doi.org/10.1073/pnas.1714282115. (In eng)

29

Building Human In Vitro 3D Models to Replace Animal Studies During Drug Discovery Research: Scientific, Ethical and Regulatory Considerations

Subrahmanyam Vangala, Uday Saxena, and C. Satish Chandran

Abstract

Novel drug discovery is a highly risky business where only 1 in 10,000–20,000 molecules successfully reaches the market. These poor success rates of new drug discovery have been attributed to poor translation of animal models to clinical outcomes. Regulatory agencies still do mandate extensive animal testing before and after a molecule enters clinical trials. Extensive efforts were made in the past three decades and revolutionary advances in (a) target-based, mechanistic models of drug discovery; (b) analytical techniques to detect the candidate drug at picomolar levels in the body fluids; (c) increased understanding of human systems biology pathways with omics platforms and other cutting-edge technologies; (d) cryopreserved banks of human primary cells; (e) digital databases that can retrieve required public information in minutes; (f) improved drug delivery technologies; (g) personalized and precision medicine tools in stratifying patient populations for clinical trial recruitments; and many more. Customized in vitro 3D models for specific clinical issues have been developed; however, they do not address the regulatory considerations to replace animals. 3D bioprinting, spheroids, and organoids of human tissues combined with microfluidics are becoming available and considerable progress has been made to mimic human physiology and drug disposition mechanisms. This review critically reviews the current state of the art of these in vitro 3D models.

Keywords

Drug discovery · Alternatives to animals · 3D bioprinting · PBPK · PK-PD · Regulatory

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29.1 Introduction

Alternatives to Animas for Drug Discovery research is influenced by several scientific, ethical and regulatory factors [1–5], as outlined in Fig. 29.1. The factors outlined in Fig. 29.1 also influence the funding sources such as Venture capitals and academic research funding sources. Our interest in Alternatives to Animals in research is purely based on scientific research experience for nearly 30 years in drug discovery and regulated drug development [6, 7]. Figure 29.1 highlights that drug discovery research is surrounded by complex ethical, regulatory, media and public opinions and, in the process, scientific rationale may be confounded. Complex scientific issues—combined with (1) ethical policies surrounding animal use; (2) ethical issues surrounding clinical trials; (3) building scientifically well thought-out alternate models to replace animals; (4) influencing regulatory agencies that alternative models are better predictive models than animals and (5) finally, convincing funding sources to conduct science-driven research—has been a huge challenge for drug discovery scientists. Considering that human safety and efficacy are the two most important criteria for registering novel drugs by regulatory agencies for human disease therapies, drug discovery researchers are faced with many challenges to discover new drugs for unmet medical needs.

A typical regulatory process (Fig. 29.2) for enabling First-In-Human (FIH) phase 1 clinical trial requires, at least a 2-week (or 4-week) GLP toxicology study using at least two animal models (Table 29.1): one small animal (rodent or mouse or rat) and one large animal (canines or non-human primates) [8]. Apart from these, multiple-dose studies, cardiovascular safety, genotoxicity and other appropriate mandated safety studies must be conducted (Fig. 29.3; Table 29.1) [8]. Additionally, in vitro

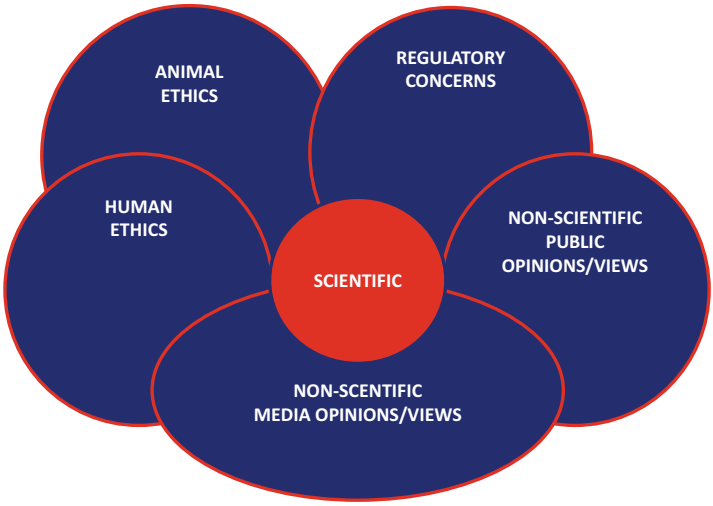

Fig. 29.1 Scientific, ethical, regulatory, and nonscientific media/public opinions surrounding drug discovery research

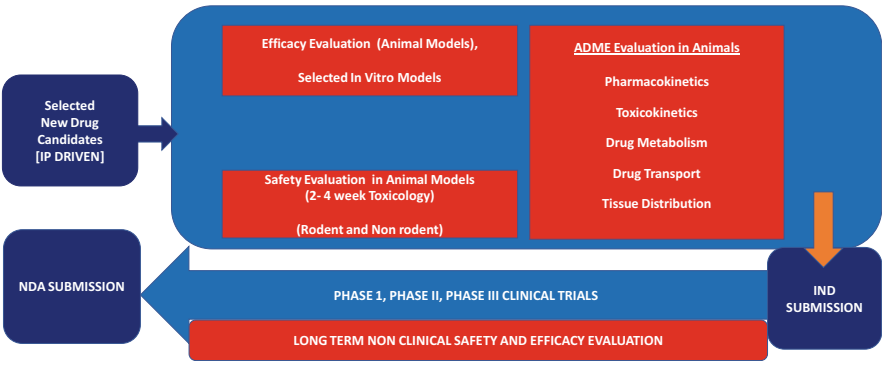

Fig. 29.2 Typical regulatory process for drug approvals involving animal studies

human ADME studies are used to incorporate appropriate scaling factors for extrapolating animal safety and efficacy data to humans. Safety margins using toxicokinetics (TK), pharmacokinetics (PK) and additional Physiologically Based Pharmacokinetic (PBPK) and Pharmacokinetic/Pharmacodynamic (PK/PD) analyses must be extrapolated for selecting a conserved FIH dose to initiate phase 1 clinical trial [7–11]. All this process is mandated by regulatory agencies with appropriate guidance documents published on agency websites (Tables 29.1 and 29.2; actual guidance documents are available at www.fda.gov). As the investigational drug proceeds through clinical phase II and phase III studies, additional, long-term safety and efficacy studies in animals are mandated by agencies that may require dosing of animals, up to a year or more (Table 29.1). In some exceptional cases, regulatory agencies are flexible to minimize the regulatory package for submissions based on unmet medical need of the disease therapy under investigation.

Agencies also require extensive reproductive toxicity studies in animals (Table 29.1), and described in detail [12]. To date, however, there are no in vitro models to replace such multi-generation repro-tox animal studies. There are some in vitro embryonic toxicity studies conducted [13]. At times, before market approval or post-approval, agencies may also request 2-year (or abbreviated 6-month) carcinogenesis studies (Table 29.1). Continued interactions with regulatory agencies help in conducting an appropriate regulatory package acceptable to agency.

Despite those extensive safety and efficacy studies that are conducted, many investigational new drugs fail during clinical trials and the approval rates by US-FDA has been stagnant [7, 15–18]. In the following, we describe the reasons "Why Drugs Fail" in clinical trials or post marketing. Table 29.3 shows percentage of failures of drugs during clinical drug development, at each stage, starting from phase I through registration by the agency [15]. Based on these failures, it is clearly evident that animal data is poorly translating into human clinical outcomes. Therefore, a scientifically reliable case is made for building physiologically relevant

Table 29.1 List of ICH guidelines on safety evaluation in animals for drug product registration at regulatory agencies[a]

• **S2(R1)** Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use
• **S3A** Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies
• **S3A** Q & As (Questions and Answers): Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure—Focus on Microsampling
• **S3B** Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies
• **S4** Duration of Chronic Toxicity Testing in Animals (Rodent and Non-Rodent Toxicity Testing)
• **S5(R2)** Detection of Toxicity to Reproduction for Medicinal Products and Toxicity to Male Fertility
• **S5(R3)** Revision of S5 Guideline on Detection of Toxicity to Reproduction for Human Pharmaceuticals
• **S5(R4)** Maintenance EWG Revision of S5 Guideline on Detection of Toxicity to Reproduction for Human Pharmaceuticals
• **S6(R1)** Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals
• **S7A** Safety Pharmacology Studies for Human Pharmaceuticals
• **S7B** The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals
• **March 2020**: Safety Testing of Drug Metabolites, Guidance for Industry—Pharmacology and Toxicology, Revision 2
• **July 2005**: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers
• **S8** Immunotoxicity Studies for Human Pharmaceuticals
• **S1(R1)** EWG Rodent Carcinogenicity Studies for Human Pharmaceuticals
• **S10** Photosafety Evaluation of Pharmaceuticals
• **S11** Nonclinical Safety Testing in Support of Development of Paediatric Medicines
• **S12** EWG Non-clinical Biodistribution Studies for Gene Therapy Products
• **S1A** Need for Carcinogenicity Studies of Pharmaceuticals
• **S1B** Testing for Carcinogenicity of Pharmaceuticals
• **S1C(R2)** Dose Selection for Carcinogenicity Studies of Pharmaceuticals
• **S9** Nonclinical Evaluation for Anticancer Pharmaceuticals
• **S9** Q & As (Questions and Answers): Nonclinical Evaluation for Anticancer Pharmaceuticals

[a] This list of ICH and/or FDA guidelines for safety evaluation covers the guidelines to uncover potential risks of genotoxicity, general organ toxicity, reproductive toxicity, cardiovascular toxicity (QT interval prolongation liability), and carcinogenicity, metabolic safety, and others as recommended by agencies

in vitro 3D models as alternatives to animal tools to translate the preclinical data for successful clinical outcomes.

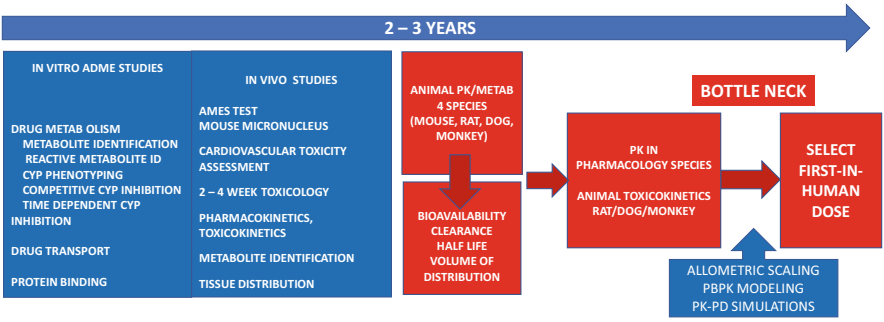

Fig. 29.3 Preclinical drug development (Regulatory mandated, GXP required)

Table 29.2 List of guidance related to clinical pharmacology and biopharmaceutics for drug product registration by agencies

• **May 2003**: Exposure-Response Relationships—Study Design, Data Analysis, and Regulatory Applications
• **March 2005**: Pharmacogenomic data submissions
• **September 2018**: Physiologically Based Pharmacokinetic Analyses—Format and Content Guidance for Industry
• **July 2019**: Population Pharmacokinetics—July 2019
• **January 2020: FDA-2017-D-5961**: In Vitro Drug Interaction Studies—Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry
• **January 2020**: Clinical Drug Interaction Studies—Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions
• **August 2020**: Drug-Drug Interaction Assessment for Therapeutic Proteins
• **September 2020**: The use of physiologically based pharmacokinetic analyses—biopharmaceutics applications for oral drug product development, manufacturing changes, and control
• **November 2020**: Clinical Drug Interaction Studies with Combined Oral Contraceptives
• **November 2020**: Evaluation of gastric pH-dependent drug interactions with acid-reducing agents: study design, data analysis, and clinical implications

29.2 Why Do Drugs Fail?

Before setting up new innovative translational models in drug discovery research, a prior knowledge of "Why do Drugs Fail?" during clinical trials or post marketing is required for alternate model building (7, 14, 15–18 and references cited therein). Such knowledge is essential to build appropriate and relevant models of interest that address the preclinical to clinical gaps and mitigate the failures. For the last three decades, extensive efforts were focused on understanding the reasons "why drugs fail?" during clinical development phases (Table 29.3). It is not only the translational issues from animals to humans, it is also translational issues from

Table 29.3 Success rates of new drug discovery in different therapeutic areas of research

Therapeutic area	Success rates (%)			
	Phase 1 to Phase 2	Phase 2 to Phase 3	Phase 3 to approval	Phase 1 nomination through approval
Ophthalmology	87.1	60.7	74.9	33.4
Vaccines (infectious diseases)	76.8	56.2	85.4	33.4
Metabolic (endocrinology)	76.2	59.7	51.6	19.6
Infectious diseases (small molecules)	70.1	58.3	75.3	25.2
Cardiovascular	73.3	65.7	62.2	25.5
Central nervous system[a]	73.2	57.9	51.1	15.0
Autoimmune diseases/ inflammation	69.8	45.7	63.7	15.1
Genito/urinary	68.7	57.1	66.5	21.6
Oncology	57.6	32.7	35.5	3.4
Overall	66.4	48.6	59.0	13.8
Overall (excluding oncology)	73.0	55.7	63.6	20.9

[a] Success rates in neurodegenerative diseases (e.g., Alzheimer's disease) are lower than oncology

healthy subjects to patients, disease subtypes, patient ethnicities and several other factors that need to be taken into account.

29.2.1 Phase I Failures

Interestingly, the early failures in phase I clinical trials are largely due to over- or underpredicting clinical PK [7] and associated safety markers extrapolated using animal models. Phase I trials, in the majority of cases, incorporate healthy subjects, not patients. Moving ahead into phase II and III trials in patients with various diseases has often met with translational issues of healthy subject data to a patient. For example, in 2017, FDA approved Midostaurin for the treatment of patients with haematological and nonhaematological malignancies, in combination with other anti-neoplastic agents [19]. However, its use is limited, as disease complications and various co-medications in patients made it difficult to perform a hepatic impairment study in the targeted patient population [20]. Metabolism and elimination of Midostaurin predominantly occurs in the liver [21]. Patients with impaired hepatic function may have a higher risk to have a decreased elimination or metabolism of Midostaurin, which may lead to increased systemic exposure or toxicity, hence understanding the impact of an impaired hepatic function on Midostaurin PK is important. Clinical pharmacologists and clinicians carefully analysed cumulative safety data from over 900 subjects exposed to Midostaurin that showed that the drug

was well tolerated in patients and in healthy subjects; thus, it was appropriate and justifiable to study Midostaurin in subjects with varying degrees of hepatic impairment. However, due to the difficulty in enrolling subjects with severe hepatic impairment, an interim analysis was performed when all mild and moderate hepatic impaired subjects, and the respective control subjects, had completed the trial, in order to obtain interim results on the PK and safety of Midostaurin in patients with mild and moderate hepatic impairment [19]. The protocol was amended in April 2018 to make the inclusion/exclusion criteria to allow enrolling the severe hepatic impairment group to the study. The final study analysis was performed when all severe hepatic impaired subjects, and the matching controls, had completed the study. This clinical trial study originally started in 2011 and was updated recently in 2021 [19]. Therefore, hepatic disease (or renal disease) can alter human PK and a very careful analysis must be performed in understanding the safety and tolerability of a new drug in treating patients with disease. The overall likelihood of approval (LOA) from Phase I for all developmental candidates was 7.9% during 2011–2020, irrespective of therapeutic area/indication [24].

29.2.2 Phase 2 Failures

As alluded to above, the failures during phase 2 trials are due to PK and safety differences between healthy subjects and patient [7, 15–18]. For example, patients do have lower expression of drug metabolizing enzymes (DME) and drug transporters (DT) due to underlying inflammation (Fig. 29.4). Extensive data is available that pro-inflammatory cytokines, growth factors do downregulate gene expression of DME and DT. Therefore, ADME data generated from healthy subjects sometimes may not be relevant to patients, and thus results in major challenges in

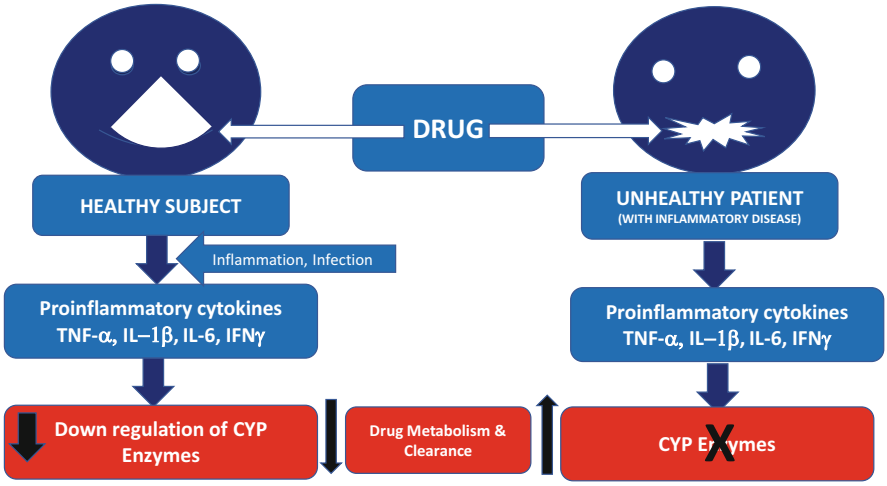

Fig. 29.4 Effect of cytokines on cytochromes P450 enzymes and drug disposition

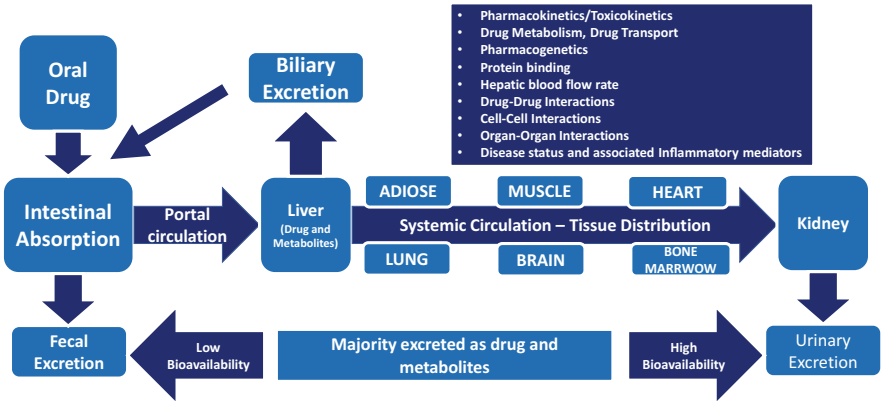

Fig. 29.5 Drug disposition and the associated factors affecting efficacy and toxicity

data extrapolation from phase 1 trials [23, 24]. Phase II development remained the largest hurdle in drug development during 2011–2020, with just 20.9% of candidates transitioning from phase I through phase III development [24] (Table 29.3).

29.2.3 Phase 3 Failures

The reason for the attrition rates of investigational drugs in phase III clinical trials appears primarily to be due to lack of efficacy (67%) and some additional safety issues. Only one in ten investigational drugs successfully pass through phase III trials and is approved by agencies. Approximately, 60% of R & D budgets are spent on clinical trials, and failures at phase III are costly failures.

29.2.4 Post-marketing Failures

Despite approvals by the agency, some drugs induce fatal and/or unmanageable idiosyncratic drug toxicities (IDTs) in humans. Between 1953 and 2013, about 462 drugs were reported withdrawn from worldwide markets and many others had black box warnings. The primary reason for these withdrawals is hepatotoxicity, and fatal cardiac arrhythmias were also reported due to drug–drug interactions (Tables 29.4, 29.5 and 29.6). Recently, ranitidine was withdrawn from the market because it spontaneously degrades to carcinogenic Nitrosamine derivatives. In 2000, troglitazone, a first-in-class PPAR-gamma inhibitor approved for type 2 diabetes, was withdrawn from the market shortly after its launch due to unpredictable idiosyncratic hepatotoxicity in patients. In some cases, there are black box warnings on many drugs, limiting their use for patient treatment. Many of these IDTs are attributed to immune system activation and auto-antibody formation against CYP enzymes and/or other proteins. The mechanism appears to be reactive metabolites

Table 29.4 Drugs withdrawn from the market due to hepatotoxicity[a]

Drug	Class	Mechanism of toxicity
Troglitazone	Antidiabetic	Reactive metabolites
Benoxaprofen	NSAID	Reactive metabolites
Bromfenac	NSAID	Reactive metabolites
Ibufenac	NSAID	Reactive metabolites
Temafloxacin	Fluoroquinolone antibiotic	Not clear
Alatrofloxacin	Fluoroquinolone antibiotic	Not clear
Trovafloxacin	Fluoroquinolone antibiotic	Mitochondrial dysfunction, inflammatory stress
Benzarone	Thrombolytic	Reactive metabolites
Ximelagatran	Anticoagulant	Immune mediated
Clomacron	Psychotropic drug	Not clear
Nefazodone	Antidepressant	Reactive metabolites
Cyclofenil	Antiestrogen	Not clear
Dilevalol	Antihypertensive	Immune mediated
Sitaxentan	Antihypertensive	Reactive metabolite, immune response
Tienilic acid	Antihypertensive	Reactive metabolites, immune response

[a] Shehu AI et al., Clin Liv Dis, 21: 35–54 (2017)

Table 29.5 Drugs currently used in the clinic that can cause hepatotoxicity[a]

Drug	Class	Pattern of injury	Mechanism of toxicity
Amoxycillin-clavulanate	Antibiotic	Cholestasis, mixed or hepatocellular	Immunoallergic
Isoniazid	Antibiotic	Hepatocellular	Reactive metabolites, immunoallergic
Ibuprofen	Analgesic	Mixed or cholestatic	Immunoallergic
Nitrofurantoin	Antibiotic	Hepatocellular	Reactive metabolites, autoimmune mediated
Propylthiouracil	Antithyroid	Hepatocellular, cholestatic or mixed	Immunoallergic
Carbamazepine	Anti-epileptic	Cholestatic, mixed or hepatocellular	Reactive metabolites, immune allergic
Valproic acid	Anti-epileptic	Mixed or hepatocellular	Mitochondrial dysfunction
Asparaginase	Anticancer	Cholestatic	Mitochondrial dysfunction
Azathioprine	Immunosuppressant	Cholestatic, mixed	Reactive metabolites, immune allergic
Infliximab	mAb for autoimmune diseases	Hepatocellular, cholestatic	Autoimmune mediated
Diclofenac	NSAID	Hepatocellular	Immunoallergic
Flutamide	Anti-androgen	Hepatocellular	Reactive metabolites

[a] Shehu AI et al., Clin Liv Dis, 21: 35–54 (2017)

Table 29.6 Some drugs withdrawn from the market due to cardiac toxicities (https://en.wikipedia.org/wiki/List_of_withdrawn_drugs)

Drug	Mechanism of toxicity
Astemizole	Fatal arrhythmias
Grepafloxacin	Cardiac repolarization and QT prolongation
Sparfloxacin	QT prolongation and phototoxicity
Sertindole	Arrythmias and sudden cardiac arrest
Terfenadine	QT prolongation and ventricular tachycardia
Terodiline	QT prolongation, ventricular tachycardia, and arrhythmias
Thioridazine	Severe cardiac arrhythmias
Chlorpheniramine	Cardiovascular toxicity
Cisapride	Fatal cardiac arrhythmias
Clobutinol	Ventricular arrhythmias and QT prolongation
Cloforex	Cardiovascular toxicity
Dexfenfluramine	Cardiotoxic
Dimethylamylamine	Cardiovascular toxicity
Dofetilide	Drug interactions, QT prolongation
Encainide	Ventricular arrhythmias
Posicor	Serious fatal drug interactions

formed during metabolism from hapten–protein conjugates, leading to immune system activation and auto-antibody generation that sometimes proved to be fatal. Figure 29.3 shows that the clinical failures are not only due to poor PK profiles in humans but also due to unpredictable IDTs in humans and not seen in animals during preclinical trials.

29.3 Key Takeaways from Failures During Drug Development

Overall rates of progression from the preclinical stage through to approvals were low. While many drivers of attrition/failures were identified, non-clinical toxicity, human pharmacokinetics and interestingly, portfolio rationalization were cited as the most common causes of failure in drug development [22, 25]. Overall, on average, it takes 10.5 years to transition a phase 1 compound into approval [24]. It appears that our success of approval rate has decreased by 25% since the 1990s [25]. Urology and Neurodegenerative diseases' therapeutic areas mark the lowest likelihood of success. The stagnant rate of drug approval rates by agencies indicate that major issue in poor success rates is not due to lack of innovation, but due to lack of translating animal data to humans for both safety and efficacy. Therefore, there is an urgent need for building Alternatives to Animal models to improve the success rate of clinical trials during drug development (Fig. 29.6).

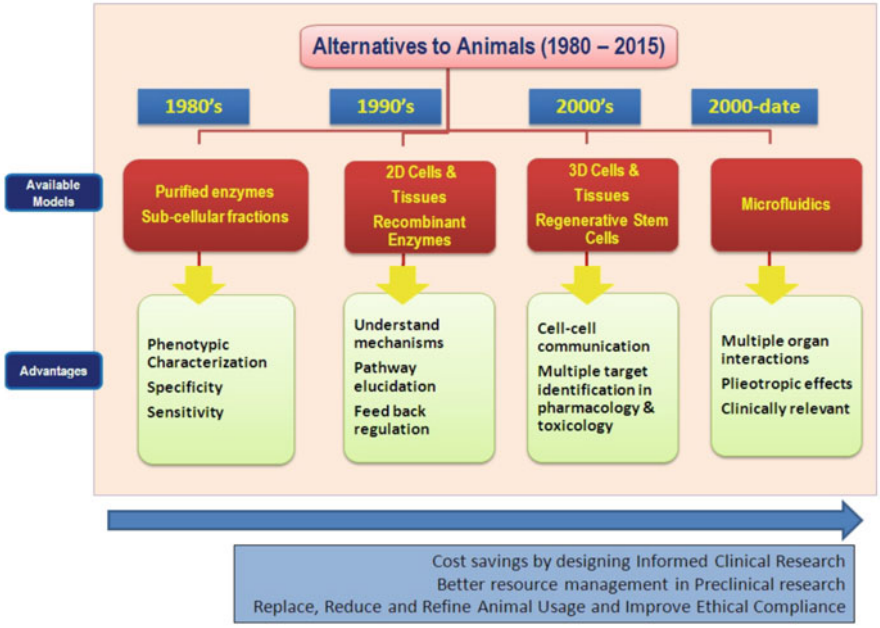

Fig. 29.6 Evolving complementary in vitro models to predict in drug efficacy and toxicity

29.4 Current Approaches of Predicting Clinical Liabilities During Preclinical Stage

Currently, many companies may use as many as four animal species such as mice, rats, canines and NHP for initial PK evaluations and conduct allometric scaling for predicting human PK (Fig. 29.3). Due to poor correlations of allometric scaling from animal PK to humans, these approaches are further refined by understanding species differences in drug metabolism (DM) and drug transport (DT) by using in vitro models of hepatocytes, liver microsomes, s-9 fractions and recombinant human cytochromes P450 (CYP), recombinant UDP-glucuronosyl transferases (UDP-GT), Caco-2 cells/MDCK cells and other transporter transfected cells, as appropriate (reviewed in [9, 10, 26–32]). Role of major human CYP enzymes (Table 29.7), uptake and efflux drug transporters (Table 29.8) present in the intestine, liver, brain and kidney, must be evaluated [7–11]. The transcriptional auto-activation of DM and DT or auto-inhibition of DM by investigational drug is evaluated for understanding and predicting changes in PK during multiple dose clinical studies. Potential drug–drug interaction (DDI) liabilities, as more and more geriatric patients are exposed to multiple drugs and metabolized by the same enzyme or transported by the same transporter, must be investigated in vitro for appropriate clinical DDI trials. Species differences in plasma protein-binding (albumin and alpha-acid glycoprotein) are also evaluated to estimate available free circulating

Table 29.7 Key human cytochrome P450 in hepatic drug metabolism

CYP enzyme	% Total (approximate)	Extent of variability (approximate)	% of drugs metabolized
1A2	12	40-fold	2
2A6	4	30–100-fold	2
2B6	<1	50-fold	2–10
2C	19	25–100	10
2D6	Up to 2.5	1000-fold	25–30
2E1	Up to 7	20-fold	4
3A4	Up to 60	90-fold	50

Table 29.8 Key drug transporters that alter PK, safety, and efficacy

ATP-binding cassette family (ABC) (Efflux) transporters	Solute carrier family (SLC) (Uptake) transporters
P-glycoprotein (P-gP; MDR1)	Organic anion transporting polypeptide (OATP1B1/1B3)
Bile salt exporter protein (BSEP)	Organic anion transporter (OAT1/OAT3)
Breast cancer resistance protein (BCRP)	Multidrug and toxin extrusion (MATE) proteins (MATE1/MATE2-K)
Multidrug resistance proteins (MRP1–MRP9)	Organic cation transporter 2 (OCT2)

drug concentrations for therapeutic activity. All this data is utilized to build physiologically-based pharmacokinetic (PBPK) modelling and using appropriate correction factors to estimate the Safe First-in-Human (FIH) Dose (Figs. 29.2 and 29.3). The pharmacodynamic (PD) data obtained in relevant animal models and the PK data obtained from those animals help in estimating PK–PD correlations and therapeutic margins at predicted pharmacologically active dose and corresponding systemic exposures.

29.5 In Vitro Human Models Available to Date

Majority of in vitro human models we use today were developed based on clinical failures either due to lack of efficacy or due to safety issues during and/or post-marketing drug recalls. Many in vitro studies using human liver surrogates such as human hepatocytes, human liver microsomes and human CYP heterologous expression systems conducted to extrapolate animal data to correct for scaling to human FIH dose. Table 29.7 shows species variation of CYP. The catalytic activities, substrate specificities and inhibitor potencies have been established for the last two decades. These properties of animal CYP are quite variable and, in many cases, metabolize drugs differently. For example [26], TPN729, a novel phosphodiesterase 5 inhibitor is metabolized to M3 (N-dealkylated metabolite of TPN729) in humans more rapidly with 7.6-fold higher exposure than that of parent drug [33]. In contrast,

in monkeys, rats and dogs, the exposure of M3 relative to TPN729 was only 3.5, 1.1 and 1.3-fold higher. This metabolite was found to be pharmacologically active at the target, thus the PK–PD effect in animals versus humans appears to be relatively higher due to M3 metabolite than TPN729 in humans compared to animals. In addition, higher exposure of M3 metabolite in humans makes the preclinical animal toxicity data questionable as M3 metabolite exposures are lower in animals. According to FDA's Metabolites in Safety Testing (MIST) guidance (Table 29.1), released in March 2020, this disproportionate metabolite ratio in humans compared to animals urges to additional toxicology evaluations to ensure this metabolite does not pose safety issues, not covered from animal toxicity studies of the parent drug.

Table 29.7 shows the variability of DM enzymes in human liver and approximate percentage of drugs that are metabolized by each CYP enzyme (reviewed in [26, 34]). CYP3A4 and CYP2D6 are the two major enzymes that catalyse the majority of marketed drugs. The population variability (1000-fold) of human CYP2D6 has been attributed to extensive intra-ethnic and inter-ethnic genetic polymorphisms of CY2D6 gene. Identifying human CYP enzymes involved in DM has now become routine practice in early drug discovery to predict potential PK differences in different intra-ethnic and inter-ethnic human populations. Knowing which CYP enzymes are involved in DM can help clinical trial designs, stratifying the patient populations via genotyping of patients. Twenty percent of drugs metabolized by CYP2D6 has been shown to have high clinical PK variability, leading to classification of human populations as Poor Metabolizers (PM), Rapid Metabolizers (RM), Extensive Metabolizers (EM) and Ultra Rapid Metabolizers (URM). Knowing CYP2D6 genotype helps in tailoring dose regimen in patients during clinical trials or post-marketing patient prescriptions. For example, Atomoxetine, a drug used for treatment of Attention Deficit Hyperactivity Disorder (ADHD), dosing adjustment is dependent on the genotype of the patient [27]. Nearly tenfold increases in systemic Atomoxetine exposure, as well as higher frequencies of adverse events in CYP2D6 PMs treated with Atomoxetine, have been confirmed, and genotyping of patients for ATM treatment was recommended when other CYP2D6 substrates are co prescribed.

CYP induction, inhibition and downregulation are also highly species-specific, and animal models are not the ideal models for assessing these properties [26, 28, 34]. CYP inhibition and CYP induction are also responsible for serious and fatal DDI, and thus regulatory agencies require these data to eliminate safety liabilities during clinical development. CYP downregulation depends on disease state due to various inflammatory mediators and has been shown to downregulate the expression of these enzymes [35, 36] (Fig. 29.4). Such differences in healthy subjects versus patients may lead to unanticipated exaggerated PK of drug that sometimes may prove to be fatal to the patient. Thus, extensive literature, for more than two decades, testifies human DM as evaluated using human in vitro tools has become the gold standard for designing human clinical trials, and endorsed by regulatory agencies [34, 37] (Fig. 29.7).

Over the last two decades, several DTs have been identified that interact with drugs in clinical use (Table 29.8). These transporters are potential barriers of entry

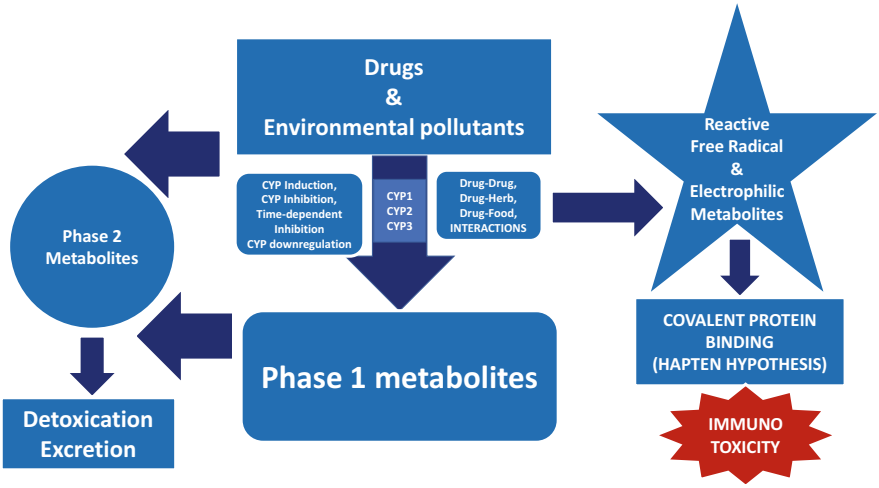

Fig. 29.7 Drug metabolism and its consequences

and efflux of many drugs, depending on their chemical properties. Some key DT are listed in Table 29.8 and are required to be investigated if they play a major role in drug disposition and uptake across the barriers. Major entry barriers include intestine, liver, brain and kidney. If a drug is not targeted for neuronal diseases, the entry of that drug in brain may have safety issues that must be eliminated, and vice versa, if a drug is targeted for neuronal diseases, its entry into brain must be characterized to ensure it reaches the target organ. In addition, due to polypharmacy in geriatric patients, whether multiple drugs which are substrates/inhibitors of the same DT may pose serious and fatal DDI and must be ruled out for all investigational drugs. FDA and other drug regulating agencies require data on some of these transporters listed in Table 29.8.

29.6 What Next in In Vitro Human Model Development?

There is no doubt that the last two decades of understanding of human ADME (in particular DM and DT) helped us to prevent serious DDI and associated safety. It also helped to pave the way to personalized approaches to treat patients such as dose tailoring based on genotype–phenotype of patient (particularly important for narrow therapeutics). It also helped in stratifying patient populations for clinical trials and increasing success in limited populations rather than the traditional "one size fits all" strategy used in clinical trials. However, there are still a lot of gaps in our understanding of human drug dispositional characteristics and we need more sophisticated tools to bridge those gaps. One major factor is that the humans are dynamic organisms with several different individual physiological factors that impact efficacy and safety in each individual. Current available in vitro human tools do not address

these dynamic factors of drug disposition. It is also true that dynamic variability of gene expression patterns of 20 major DM and DT proteins, is the future challenge with current in vitro human ADME tools. Additional challenges include [1] drug and its metabolites do not stay in liver, the primary site of metabolism, but circulate into many other highly vascularized tissues, including lung, brain, kidneys, muscle, adipose, etc. Blood flow rates, plasma protein binding, the secondary extrahepatic metabolism of the drug cannot be evaluated by only using human hepatocytes or its subcellular fractions. Dynamic interaction of cytokines and growth factors secreted by different cell types in each organ (epithelial, endothelial, endogenous macrophages, etc.), hormonal and diurnal variations in basic biochemical systems influence the expression and activities of most DM and DT protein in liver and other organs. Therefore, advanced sophisticated and customized in vitro tools that integrate dynamic interactions of liver with rest of the body are required for improving human successful clinical outcomes during clinical drug development.

29.7 Use of In Vitro 3D Human Models: Next Paradigm Shift?

In the last two decades, there have been extensive efforts in building human in vitro 3D models to address the gaps described above [7, 38–41]. However, the progress has been very slow due to lack of primary cells of different organs of human origin. Commercial, cryopreserved banks of all types of cells of human organs are rapidly being made available for building Disease-in-a-Dish and Organ-on-a-Chip, human in vitro models [42]. Some highly ambitious industrial researchers (e.g. Tissues and CSEM) are building customized Human-on-a-Chip models [43] and validating them for predictive PBPK, PK–PD, Toxicology and Efficacy of drugs.

Regulatory agencies are also on board by validating 3D microphysiological systems [43] and entering into agreements with private industries [44–46]. In addition, FDA Modernization act of 2021 can leverage technology to accelerate alternatives to animals in drug discovery and development research to save millions of animals [47]. The data generated from these models can improve the existing databases and AI models, which were originally built from published literature mostly of animal origin. Our vision is integrating human in vitro 3D models with PBPK and PK–PD evaluations and build databases for tailored clinical trial designs in human populations (Fig. 29.8). These models eventually can get regulatory blessings to replace animal models, which are not well translated for successful clinical outcomes in humans.

29.8 Learnings from Environmental Toxicology

Many environmental pollutants have been shown to be one of the major causes of increased cancers and other toxic effects. The risk assessment of several environmental pollutants and setting their exposure limits in our atmosphere and for occupational exposures has been a challenge for many decades. The learnings

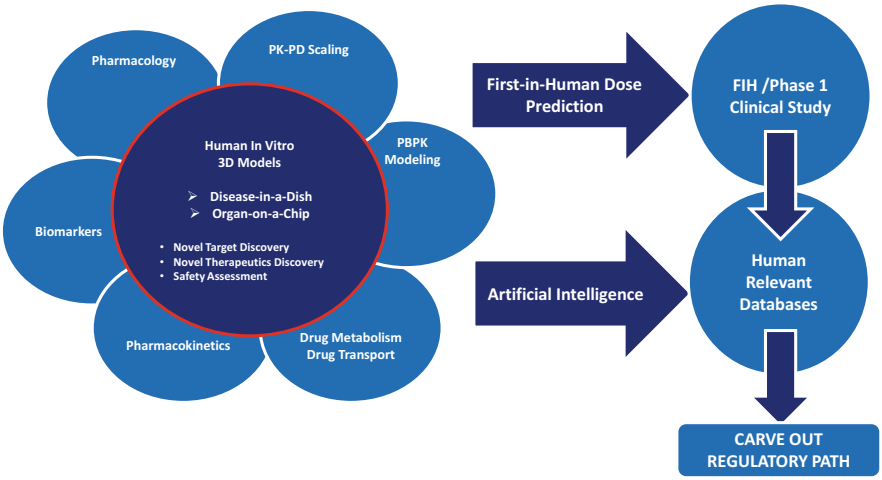

Fig. 29.8 New drug discovery—integrated and customized 3D human models—another paradigm shift?

from safety evaluations and associated regulatory framework are extremely useful for decision-making processes and in vitro/in silico predictive model building.

Environmental Protection Agency (EPA) sets guidelines and exposure limits based on the ongoing research activities at academic institutes and government health institutes. Toxicology in the twenty-first century (Tox21) is a unique collaboration between several federal agencies to develop new ways to rapidly test whether chemical substances in our daily environment adversely affect human health [48]. Chemical substances assayed in Tox21 include a diverse range of products such as: commercial chemicals, pesticides, food additives/contaminants and medicinal compounds, including drugs and medical devices. Four primary organizations that collaborated as part of Tox21 include:

- **National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP)**, National Institutes of Health (NIH), U.S. Department of Health and Human Services
- **National Center for Advancing Translational Sciences (NCATS)**, National Institutes of Health (NIH)
- **U.S. Food and Drug Administration (FDA)**, U.S. Department of Health and Human Services
- **National Center for Computational Toxicology**, Office of Research and Development, U.S. Environmental Protection Agency (EPA)

The NTP interagency center for the Evaluation of Alternative Toxicological Methods (NICEATM) focused on the development and evaluation of Alternative Test Methods to comply with 3R guidelines. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is a permanent

committee of NIEHS formally established in 2000. ICCVAM is composed of representatives from 17 U.S. federal regulatory and research agencies. These regulatory and research agencies require, use, generate or disseminate toxicological and safety testing information. ICCVAM facilitates interagency and international collaborations promoting the development, regulatory acceptance and use of alternative tests that encourage the reduction, refinement or replacement of animal test methods. ICCVAM also provides guidance to researchers interested in developing new test methods. They evaluate recommendations from expert peer reviews of alternative toxicity test methods and report to appropriate federal agencies. Although ICCVAM governs all three of the 3Rs, in 2018, it published a strategic plan to replace the use of animals in testing. The new roadmap incorporates and recommends REPLACING ANIMAL TESTS with NON-ANIMAL approaches. In 2019, EPA announced that it is aggressively pursuing use of non-animal alternatives and committed to eliminate use of animals by year 2035.

National Toxicology Program (NTP) has instituted research on identifying Adverse Outcome Pathways (AOP) for building new alternate in vitro models and/or using computational models with available published literature [49]. The main goal of identifying AOPs is to identify the "Key Molecular Events" in toxicity pathways. Use of 3D in vitro human models is highly encouraged by NTP and, currently, many organizations are conducting research on identifying AOPs which can provide a conceptual basis for non-animal testing strategies. Integrated 3D in vitro human models appear to be on top of the list for identifying AOPs for many environmental toxicants that adversely affect human health.

In addition to AOPs, several academic researchers have identified "Key Characteristics (KC)"(Tables 29.9, 29.10, and 29.11) of chemical carcinogens, cardiovascular toxins, male and female reproductive toxins, endocrine disruptors, etc. These KCs could also become guides for identifying KMEs in AOPs. Developing alternative in vitro human surrogate 3D models should be applied for identifying more KCs and KMEs.

29.9 Carving Out a Regulatory Path

FDA, EPA and other regulatory agencies have been quite positive and cooperative in encouraging industry to develop translational models that bridge the gaps between regulatory clinical drug development and reduce clinical attrition rates. FDA's modernization act of 2021 has introduced a bill to amend the Federal Food, Drug, and Cosmetic Act to allow manufacturers and sponsors of a drug to use alternative testing methods to animal testing to investigate the safety and effectiveness of a drug, and for other purposes. (FDA Modernization Act of 2021 (S. 2952)—GovTrack.us). In accordance with this, recently, FDA announced a collaboration with CN-Bio on using lung-on-a-chip model and its usefulness to evaluate efficacy and safety of inhaled drugs (CN Bio FDA collaboration expanded (cn-bio.com)). Previously, CN-Bio and FDA co-published an article on reproducibility of liver microphysiological system for evaluating drug toxicity, metabolism and

Table 29.9 Key characteristics of chemical carcinogens (Smith MT et al, Environ Hlth Perespect., 124(6):713–721 (2016))

	Key characteristics	Examples of relevant streams of mechanistic evidence
KC1	Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
KC2	Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
KC3	Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base excision, or double-strand break repair)
KC4	Induces epigenetic alteration	DNA methylation, histone modification, microRNA expression
KC5	Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
KC6	Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine, and/or chemokine production
KC7	Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
KC8	Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
KC9	Causes immortalization	Inhibition of senescence, cell transformation
KC10	Alters cell proliferation, cell death, or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

accumulation. FDA also announced collaboration with Emulate, using lung-on-a-chip model for evaluating safety and protective immunity of Covid-19 vaccines.

The poor predictive nature of animal models for clinical translation prompted the pharma industry to adopt relevant and appropriate in vitro human models for improved translation of animal data. In some cases, humanized animal models containing human genes in place of animal gene have been used. However, success is still limited and many gaps still perplex the drug discovery researcher. The need for more human relevant models is required and must be continued. In the following, we describe available human in vitro models and their current successful clinical translation, followed by future innovation to bridge the existing gaps in clinical translation.

29.10 Conclusions

For any disease, it is difficult to discover effective and safe drugs. Discovering and developing a successful drug depends on very detailed knowledge of underlying disease mechanisms and a successful progression from candidate identification to

Table 29.10 Key characteristics of endocrine disrupting chemicals (La Merrill MA ete al, 16(1): 450–47 (2020))

	Key characteristic	Examples of relevant streams of mechanistic evidence
KC1	Interacts with or activates hormone receptors	Binding or agonism of hormone receptors
KC2	Antagonizes hormone receptors	Antagonism of nuclear or cell surface hormone receptors
KC3	Alters hormone receptor expression	Abundance, distribution, and degradation of hormone receptors
KC4	Alters signal transduction in hormone-responsive cells	Abundance of post-translational modifications, cofactors, transcription factors and transcripts, and activity of associated enzymes
KC5	Induces epigenetic modifications in hormone-producing or hormone-responsive cells	Chromatin modifications, DNA methylation, and noncoding RNA expression
KC6	Alters hormone synthesis	Expression or activity of enzymes or substrates in hormone synthesis
KC7	Alters hormone transport across cell membranes	Intracellular transport, vesicle dynamics, or cellular secretion
KC8	Alters hormone distribution or circulating hormone levels	Blood protein expression and binding capacity, blood levels of pro-hormones and hormones
KC9	Alters hormone metabolism or clearance	Inactivation, breakdown, recycling, clearance, excretion, or elimination of hormones
KC10	Alters fate of hormone-producing or hormone-responsive cells	Atrophy, hyperplasia, hypertrophy, differentiation, migration, proliferation, or apoptosis

optimal clinical trial design. The pharmaceutical industry (for all the right reasons) is heavily regulated, and it is one of the few industries where, despite the investment of a great deal of capital and time, the majority of efforts result in complete failure. While other industries (e.g, aircraft), are equally well regulated, the result of that scrutiny is rarely a completely unusable aircraft, or the irreversible denial of marketing approval for a new airplane to be launched into market. We understand enough about aeronautics to assure that planes will fly, and an iterative process with regulators makes sure they fly safely. In the discovery and development of new medicines, the complexity of human biology and inter- and intra-individual differences are such that "one size fits all" doesn't work. We do not have any a priori reason to expect that we can intervene with pharmaceutical agents in any disease, and it is never assured that a drug will be approved for marketing. Therefore, drug deelopment is a continuously evolving process of paradigm shifts. As new incremental innovations and technologies emerge, our improved understanding of complex pathways of human disease pathology will help us develop novel therapeutics for human diseases.

Table 29.11 Key characteristics of cardiovascular toxicants (Lind L et al, Environ. Hlth. Perspect. 129(9):095001 (2021))

	Key characteristics	Examples of relevant streams of mechanistic evidence
KC1	Impairs regulation of cardiac excitability	QTc prolongation in humans
KC2	Impairs cardiac contractility and relaxation	Alters calcium homeostasis by inducing calcium leakage from the sarcoplasmic reticulum
KC3	Induces cardiomyocyte injury and death	Induces cardiomyocyte apoptosis, necrosis, necroptosis, and autophagy in cardiac cells
KC4	Induces proliferation of valve stroma	In vitro activation of 5-HT2B receptors; development of valvular cardiac disease in clinical studies
KC5	Impacts endothelial and vascular function	
KC6	Alters hemostasis	
KC7	Causes dyslipidemia	
KC8	Impairs mitochondrial function	Promotes mitochondrial fission, inhibits mitochondrial fusion, and impairs mitochondrial function in several ways, including decreasing the oxygen consumption rate and altering mitochondrial membrane potential
KC9	Modifies autonomic nervous system activity	
KC10	Induces oxidative stress	
KC11	Causes inflammation	Chronic environmental exposures are associated with elevated circulating inflammatory markers in humans

The past three decades witnessed an explosion of technologies (described in the abstract) and we understand the disease processes better with new advanced models that are at our disposal. We are no longer dependent on animal models to intervene in the disease process to develop new drugs. We are at a crossroads where we have several human tissue banks, primary cell banks and human stem cells. With these technologies, coupled to advanced analytical technologies and micro-/macrofluidics, we can create mini human chips that can be used to understand the human disease processes and, at the same time, discover novel targets for disease intervention with novel therapeutics. We propose that regulatory agencies, academics and industries work together toward a common goal of developing successful drugs for human diseases (not animal diseases).

1. In vitro 3D models can help in elucidating the entire sequence of biological pathways that adversely affect human health. Disease-in-a-Dish models can help discover novel human druggable targets using these tools.
2. In vitro 3D models can be optimized to reflect human drug disposition mechanisms that are relevant to humans, not animals. Customized animal/human-on-a-chip models can help mimic human physiology and enable PBPK and PK–PD models become more relevant to humans (unlike animal models).

3. Mini-clinical trials (clinical trials in a dish), using patient biopsies, can be advanced in in vitro 3D models that can help design robust human clinical trials starting with FIH trials with more successful translational outcomes through phase II and phase III.
4. Finally, using micro-/macrofluidics, we need only grams of quantities of drugs for these evaluations, unlike animal studies that require kilograms of drug batches.

In a nutshell, all these advances will also decrease exorbitant costs of drug development with decreased attrition rates and more drugs for serious unmet medical needs. Science, coupled to ethics and regulations, should take priority more than media/public opinions. Angel investors, venture capitals government funding agencies should facilitate increased funding for research on human 3D in vitro models to enable applying these cutting edge technologies to discover novel human disease targets and therapies.

References

1. Hägglöf I, Holmgren Å (2012) Chapter 20 - Regulatory affairs. In: Hill RG, Rang HP (eds) Drug discovery and development, 2nd edn. Elsevier, Amsterdam, pp 285–301
2. Moyer ED (2006) Chapter 12 - Regulatory affairs: keeping product development on track. In: Robbins-Rot C (ed) Alternative careers in science, 2nd edn. Elsevier, Amsterdam, pp 125–138
3. Ethical Issues in Drug Development | Stanford eCorner (2011)
4. Preziosi P (2004) Science, pharmacoeconomics and ethics in drug R & D: a sustainable future scenario? Nat Rev Drug Discov 3:521–526
5. Klein AV. Ethics in Clinical Trials and Drug Development. Ready to fulfil its Destiny? Ethics in Clinical Trials and Drug Development (pharmafocusasia.com)
6. Saxena U (2010) Alzheimer's disease amyloid hypothesis at crossroads: where do we go from here? Expert Opin Ther Targets 14(12):1273–1277
7. Vangala S et al (2012) Translational drug discovery research: integration of medicinal chemistry, computational modeling, pharmacology, ADME, and toxicology. In: Lyubimov AV (ed) Encyclopedia of drug metabolism and interaction; Part III. General principles of ADME. Drug transporters and other mechanisms of transport. Wiley, Hoboken, NJ
8. Stark C, Steger-Hartmann T (2016) Nonclinical safety and toxicology. Handb Exp Pharmacol 232:261–283
9. Eichenbaum G, Vangala S, Tonelli AP (2010) Toxicokinetics in support of drug development. In: Cayen MP (ed) Early drug development: strategies and routes to first in human clinical trials. Wiley, Hoboken, NJ, pp 309–359
10. Vangala S, Tonelli AP (2005) Chapter 4: Pharmacokinetics/ADME of small molecules. In: Rogge MC, Taft DR (eds) Preclinincal drug development, 1st edn. CRC Press, Boca Raton
11. Maeng H-J, Chow ECY, Fan J, Pang KS (2012) Physiologically based pharmacokinetic (PBPK) modeling: usefulness and applications. In: Lyubimov AV (ed) Encyclopedia of drug metabolism and interactions; Part III. General principles of ADME. Drug transporters and other mechanisms of transport. Wiley, Hoboken, NJ
12. Wangikar P, Ahmed T, Vangala S (2011) Chapter 76: Toxicologic pathology of reproductive system. In: Gupta RC (ed) Reproductive and developmental toxicology, 1st edn. Elsevier, Amsterdam, pp 1003–1026
13. Popov V, Protasova G (2011) Chapter 12: In vitro embryotoxicity testing. In: Gupta RC (ed) Reproductive and developmental toxicology, 1st edn. Elsevier, Amsterdam, pp 147–157

14. Pellicciari R (2017 June) Attrition in the pharmaceutical industry: reasons, implications, and pathways forward. In: Alex A, Harris JC, Smith DA (eds). Wiley, Hoboken. ChemMedChem 12(13)
15. Wong CH, Siah KW, Lo AW (2019) Estimation of Clinical trial success rates and related parameters. Biostatistics 20(2):273–286
16. Dowden H, Munro J (2019) Trends in clinical success rates and therapeutic focus. Nat Rev Drug Discov 18:495–496
17. Batta A, Kalra BS, Khirasaria R (2020) Trends in FDA drug approvals over last 2 decades: an observational study. J Family Med Prim Care 9(1):105–114
18. Waring JM et al (2015) An analysis of the attrition of drug candidates from four major pharmaceutical companies. Nat Rev Drug Discov 14:475–486
19. Midostaurin | FDA. April 28, 2017.
20. PK and Safety of Midostaurin in Subjects With Impaired Hepatic Function and Subjects With Normal Hepatic Function - Full Text View - ClinicalTrials.gov; Recruitment Status: First Posted: September 7, 2011; Last Update Posted: May 12, 2021.
21. Dutreix C, Munarini F, Lorenzo S, Roesel J, Wang Y (2013) Investigation into CYP3A4-mediated drug–drug interactions on midostaurin in healthy volunteers. Cancer Chemother Pharmacol 72:1223–1234
22. Palatini P, De Martin S (2016) Pharmacokinetic drug interactions in liver disease: an update. World J Gastroenterol 22(3):1260–1278
23. Yee KL, Mengyao Li M, Cabalu T, Sahasrabudhe V, Lin J, Zhao P, Jadhav P (2018) Evaluation of model-based prediction of pharmacokinetics in the renal impairment population. J Clin Pharmacol 58(3):364–376
24. Clinical Development Success Rates and Contributing Factors 2011–2020. 2021-clinical-development-success-rates-2011-2020-v17.pdf (informa.com).
25. Three ways to overcome attrition in NCE development | CAS. February 16, 2021.
26. Manikandan P, Nagini S (2018) Cytochrome P450 structure, function and clinical significance: a review. Curr Drug Targets 19(1):38–54
27. Yu G, Li G-F, John S, Markowitz JS (2016) Atomoxetine: a review of its pharmacokinetics and pharmacogenomics relative to drug disposition. J Child Adolesc Psychopharmacol 26(4): 314–326
28. Clarke SE, Jones B (2008) Human cytochromes P450 and their roles in metabolism-based drug-drug interactions. In: Rodriguez AD (ed) Drug-drug interactions. Informa, New York, pp 53–86
29. Remmel RP, Zhou J, Argikar UA (2008) UDP-glucuronosyltransferases. In: Rodriguez AD (ed) Drug-drug interactions. Informa, New York, pp 87–134
30. Kusuhara H, Sugiyama Y (2008) Drug-drug interactions involving the membrane transport process. In: Rodriguez AD (ed) Drug-drug interactions. Informa, New York, pp 135–204
31. Silva JM, Nicoll-Griffith DA (2008) In vitro models for studying induction of cytochrome P450 enzymes. In: Rodriguez AD (ed) Drug-drug interactions. Informa, New York, pp 205–230
32. Ogilvie BW, Usuki E, Yerino P, Parkinson A (2008) In vitro cytochrome P450 inhibition data and the prediction of drug-drug interactions: qualitative relationships, quantitative predictions, and the rank-order approach. In: Rodriguez AD (ed) Drug-drug interactions. Informa, New York, pp 231–358
33. Tian Q-Q, Zhu Y-T, Diao X-X, Zhang X-L, Xu Y-C, Shen J-S, Wang Z, Zhong D-F (2020) Species differences in the CYP3A-catalyzed metabolism of TPN729, a novel PDE5 inhibitor. Acta Pharmacol Sinica 0:1–9
34. Drug Development and Drug Interactions | Table of Substrates, Inhibitors and Inducers | FDA. March 10, 2020.
35. Morgan ET (2001) Regulation of cytochrome p450 by inflammatory mediators: why and how? Drug Metab Dispos 29(3):207–212
36. Renton KW (2004) Cytochrome P450 regulation and drug biotransformation during inflammation and infection. Curr Drug Metab 5(3):235–243

37. Guidance for Industry (fda.gov). Guidance for Industry: In Vitro Drug Interaction Studies Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions. Clinical Pharmacology. January 20, 2020.
38. Joseph X, Akhil V, Arathi A, Mohanan PV (2022) Comprehensive development in organ-on-a-chip technology. J Pharm Sci 111(1):18–31
39. Saxena S. Meher K, Rotella M, Vangala S, Chandran S, Malhotra N, Palakodeti R, Voleti SR, Saxena U (2021) Identification of SGLT2 inhibitor Ertugliflozin as a treatment for COVID-19 using computational and experimental paradigm. bioRxiv 2021.06.18.448921. https://doi.org/10.1101/2021.06.18.448921
40. Saxena S. Meher K, Rotella M, Vangala S, Chandran S, Malhotra N, Palakodeti R, Voleti SR, Saxena U (2021) Tyrosine kinase inhibitor family of drugs as prospective targeted therapy for COVID-19 based on in silico and 3D-human vascular lung model studies. bioRxiv 2021.05.13.443955. https://doi.org/10.1101/2021.05.13.443955
41. Saxena S, Meher K, Rotella M, Vangala S, Chandran S, Malhotra N, Palakodeti R, Voleti SR, Saxena U. *In silico* and *in vitro* demonstration of homoharrintonine's antagonism of RBD-ACE2 Binding and its anti-inflammatory and anti-thrombogenic properties in a 3D human vascular lung model. bioRxiv 2021.05.02.442384. https://doi.org/10.1101/2021.05.02.442384
42. Novabiosis | Primary Human Cells
43. Rubiano A, Indapurkar A, Yokosawa R, Miedzik A, Rosenzweig B, Arefin A, Moulin CM, Dame K, Hartman N, Volpe DA, Matta MK, Hughes DJ, Strauss DG, Kostrzewski T, Riberio AJS (2021) Characterizing the reproducibility in using a liver microphysiological n system for assaying drug toxicity, drug metabolism, and accumulation. Clin Transl Sci 14(3):1049–1061
44. Multi Organ Chip, automated MOC measures (gateone-project.eu)
45. FDA signs collaborative agreement with CN Bio Innovations to use Organs-on-Chips to improve drug development and evaluation. (CNbio.com) October 26, 2017.
46. Emulate Signs Collaborative Agreement with the FDA to Apply Lung-Chip to Evaluate Safety of COVID-19 Vaccines and Protective Immunity Against SARS-CoV-2 | Emulate (emulatebio.com), October 29, 2020.
47. FDA Modernization Act 2021. H.R.2565 - 117th Congress (2021–2022): FDA Modernization Act of 2021 | Congress.gov | Library of Congress. April 15, 2021.
48. Toxicology in the 21st century. Tox21 - Toxicology in the 21st Century
49. OECD Series on Adverse Outcome Pathways | OECD iLibrary (oecd-ilibrary.org)