Tissue Chips: Contemporary Applications and Advancements

17

Taranjot Kaur, Jigyasa Sharma, and Seemha Rai

Abstract

The tissue chip is an efficient and highly applicable novel product developed by the amalgamation of cell biology, engineering and biomaterial technology. It is a functional organ biomimic model built on a dynamic microfluidic chip that replicates the architecture, physiology and functionality of an organ as observed in vivo. This tissue chip technology has the potential to be utilized for drug screening and safety testing during preclinical trials. The current preclinical studies use the 2-dimensional cell culture and animal models, which do not always translate into successful clinical trials. The tissue chip or micro physio-logical system is a far more efficient preclinical model as it is designed to mimic the human organ system in its true sense, thus increasing the success rate during human clinical trials. It also holds the potential to completely eliminate the utilization of animals as drug testing models. These systems can also be used to model diseased state, thus providing a stronger apprehension of the mechanisms behind disease pathologies and development of new therapeutic agents. This technology is the future of personalized medicine as diseased cells from the patient can directly be used to understand the disease mechanism and to develop a novel treatment for the same. This tissue chip technology has surfaced in the past two decades and has recently been selected by the global economic forum as one of the top ten developing technologies. The article will discuss the various contemporary applications of the tissue chip technology, including its use in the testing for suitable drugs against the current novel coronavirus. Although, there are still a few limitations to the organ-on-a-chip technology, it is continuously evolving as a potential model for translational research and precision medicine.

T. Kaur · J. Sharma · S. Rai (✉)
Centre for Stem Cell Tissue Engineering and Biomedical Excellence, Panjab University, Chandigarh, Punjab, India

253

17.1 Introduction

17.1.1 What Is a Tissue Chip?

Tissue chips or more commonly known as organ-on-a-chip (OOAC) are the devices that reiterate the structure, role and physiology of the organs of our body. These micro physiological systems (MPS) are designed in such a manner that allows them to position the cells (mostly stem cells) in a structurally accurate 3-dimentional (3D) formation that recapitulates the function and physiology of the various organs of the body and also elicits a response to various stimuli such as interaction with drugs, hormones, cell signalling molecules and biomechanical stimuli (Low and Tagle 2017). The major goal of an OOAC, as a biomimic apparatus, is to imitate the comprehensive environment of the human organ (Bhatia and Ingber 2014a) and simulate a biologically accurate response to external stimuli, hence bridging the gap between in vivo and in vitro data (Fig. 17.1) (Wu et al. 2020; Ramadan and Zourob 2020). These tissue chips are based on the microfluidic technology. Microfluidics is a branch of science that exactly controls and processes (10^{-9} to 10^{-18} L) fluids in the micro range (Wu et al. 2020).
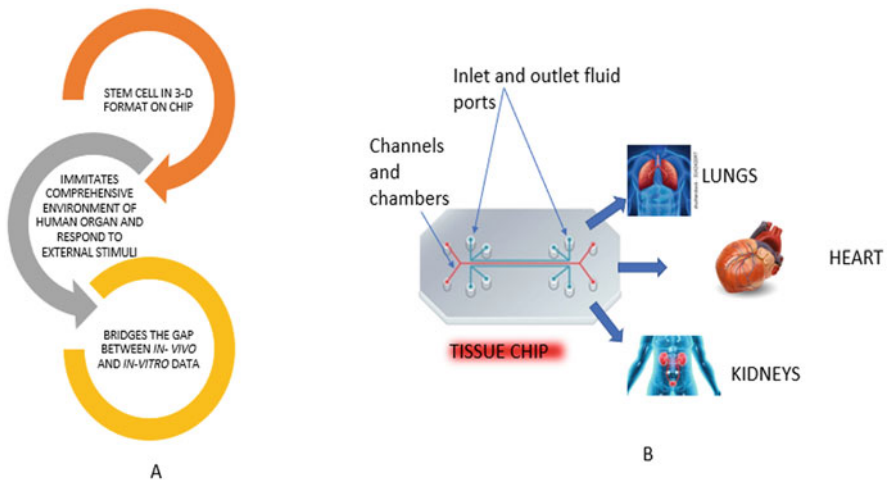
Fig. 17.1 (**a**) Major goal of an OOAC technology. (**b**) OOAC apparatus imitating the comprehensive environment of the human organ

17.1.2 Why Do We Need Tissue Chips?

It has been clearly observed that most of the newly developed drugs do not progress beyond phase 2 and phase 3 clinical trials. The average cost involved in the research and development of a new medicine is estimated to be around $2.6 billion by PhRMA (Pharmaceutical Research and Manufacturers of America). It has also been reported that for a medicine to gain approval, it takes at least 10 years starting from the initial discovery stage. The clinical success is achieved by those drugs that are able to enter the clinical trials stage, and this amounts to 12% (Scannell et al. 2012; Tufts 2014). Once a lead compound for a particular biological target has been discovered, it has to be analysed on the basis of its pharmacological properties to be able to be tested on preclinical models (Low and Tagle 2017; Mittal et al. 2019).

Currently, 2D cell cultures and animal models are being utilized as drug screening models at preclinical level. Although both these methods have some level of credibility, neither of these models adequately mimic the human physiological responses against the drug being tested (Fig. 17.2a). This leads to a higher rate of failure of drugs at a clinical trial level. Therefore, there is a need for a model system that accurately depicts the structural and functional heterogeneous environment of multiple cell types and their interactions in a human organ in 3D (Fig. 17.2b). Hence, OOAC is the required predictive tool for risk evaluation in the process of developing a drug and is a unique platform to help potent treatments succeed with more assurance (Fig. 17.2c) (Coller and Califf 2009).

Organs-on-chips are being used as tools for choosing the drug and checking the toxicity. The creation of neural microsystems has highlighted examples of tissue chip's potential use for checking the toxicity (Schwartz et al. 2015), which will be discussed in the later sections of this article. Tissue chips can also be used as an excellent platform for disease modelling. This technology is highly helpful for the patients suffering from rare diseases. It provides new ways for understanding pathologies and to discover potent therapeutics for various disorders. Diseases could be modelled on the chip using stem cells of the patient donors to induce a diseased phenotypic condition that can be observed in vitro (Azizipour et al. 2020; Low and Tagle 2016; Chen et al. 2020; Marx et al. 2016).

17.1.3 Tissue Chips: Preparation and Design

The entire process of preparation of OOAC is called soft lithography. Soft lithography is the technology used to fabricate substances composed of elastomeric materials. The first step of the process consists of photolithography of a silicon wafer which then produces a negative copy of the desired mould (Huh et al. 2013). The photoresist material is then put on the top of the silicon wafer, and uncovered region is etched by ultraviolet light. Subsequently, polydimethylsiloxane (PDMS) is dispensed into this mould, which thus creates a positive copy of the desired design. Then, this PDMS copy is sealed to a glass slide, forming closed-circuit channels (Fig. 17.3) (Bhatia and Ingber 2014b). The chip is now ready to be connected to
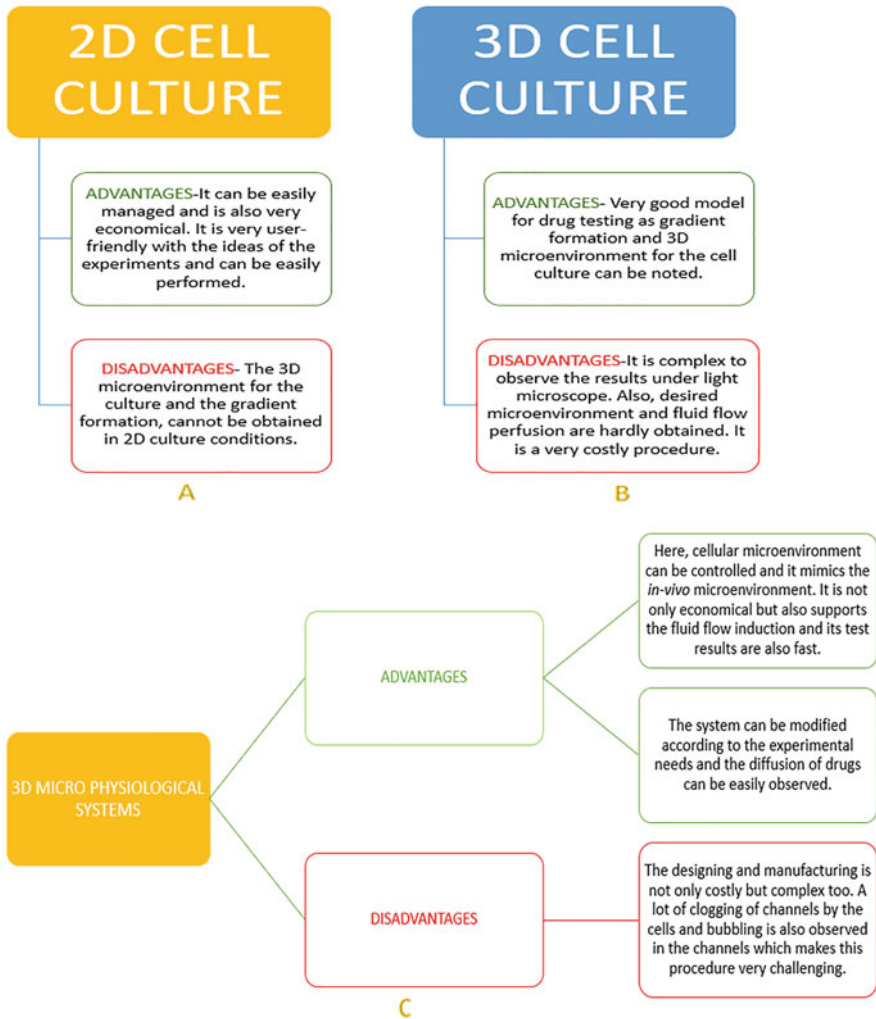
Fig. 17.2 (**a**, **b**, **c**) Comparison between the advantages and disadvantages of macroscopic 2D and 3D cell culture models and micro physiological cell culture platforms

microfluidic reservoirs and pumps using microfluidic tubing. PDMS is biocompatible, easy to mould and is transparent, therefore allowing for easy representation with photography. This makes PDMS optimum to be used in organ miniaturization (Huh et al. 2013; Bhatia and Ingber 2014b).

Further designing and mechanism of the tissue chips are dependent on the following key components:

- *Fluid shear force*: The cells cultured in a dynamic nature through micropump perfusion is enabled through microfluidics. It aids the management of nutrients
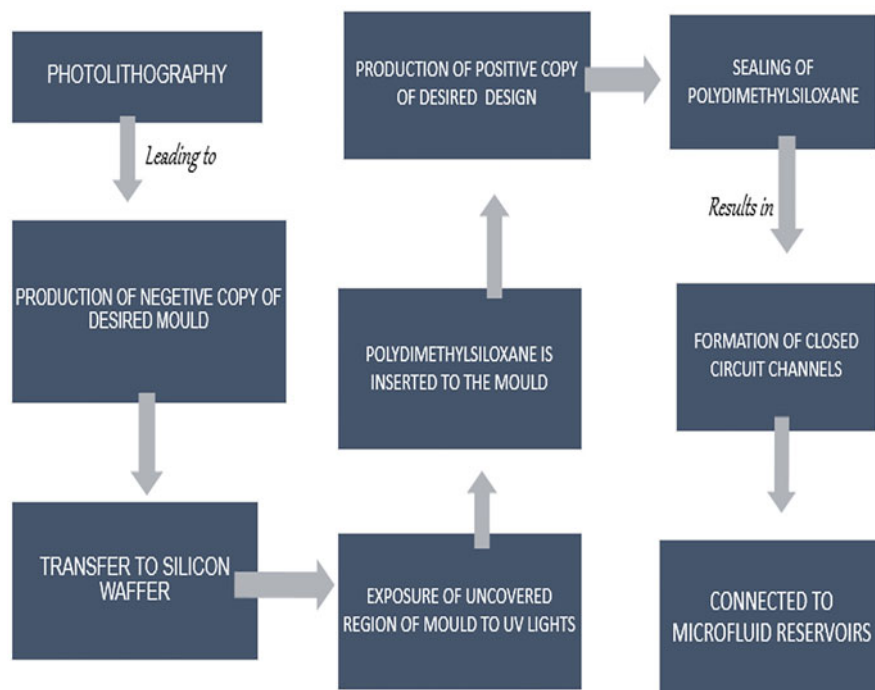
Fig. 17.3 Flow chart representing the process of *Soft Lithography*

and timely waste release on the chip. This microfluidic potent environment of the chip resembles the in vivo conditions. Also, the organ polarity is caused by fluid shear stress (Theobald et al. 2018). The flow to the organ chip system is ensured through a simple "rocker" on a chip fluid motion. The flow can also be ensured with the help of a "pulsatile" set-up (Wu et al. 2020; Ronaldson-Bouchard and Vunjak-Novakovic 2018).

- *Concentration gradient*: The fluid in the organ chips acts as a laminar flow. It leads to the flow of biological molecules in the form of a stable gradient and controls it both with regard to space and time at a microscale level. Various concentration gradient-driven biochemical signals exist in biological phenomenon such as angiogenesis, invasion and migration (Wu et al. 2020; Yang et al. 2014; Nguyen et al. 2013; Song et al. 2012).

- *Dynamic mechanical stress*: Organ pressures inside the body such as lung pressure, blood pressure and bone pressure are responsible for the maintenance of the mechanically stressed tissues (skeletal muscle, bone, cartilage, etc.) in the body (Sato and Clevers 2013; Sellgren et al. 2015). Using microfluidics, elastic porous membranes are used on the MPS to create periodic mechanical stresses. These mechanical stimulations are a major factor in determination of differentiation of cells during physiological processes on the chip (Yang et al. 2017; Kshitiz et al. 2012).
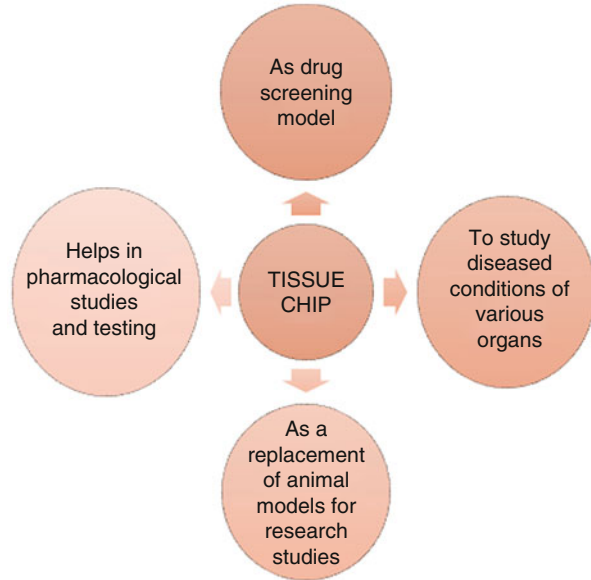
• *Cell patterning*: In vitro micro physiological models have complex geometries. The cell patterning for these micro physiological models is controlled by microfluidics. Cell patterning on the chip is contributed by surface modifications, templates and 3D printing (Zhou et al. 2012; Tibbe et al. 2018; Xue et al. 2018). The use of 3D printing allows and enables the development of user-defined customized moulds to enhance adaptability and utility in cell patterns which are essential to recapitulate the cellular microenvironment on the tissue chip.

Tissue chips have extensive usage in drug screening and disease modelling areas (Fig. 17.4). Some of the recent applications are discussed in the following sections.

17.2 Tissue Chips as Drug Screening Model for Coronavirus and Other Viruses of the Respiratory Tract

In the light of the recent events, it has been proven that a respiratory tract virus such as SARS-CoV-2 can create havoc in the entire world and lead to a global pandemic. Respiratory tract viruses such as influenza and coronavirus are a major threat to public health and world economy. The pandemic Spanish flu in 1918, which developed due to H1N1 influenza A virus infection, is estimated to have been responsible for the deaths of 50–100 million people and lasted for 2 years. These rapidly spreading virus pandemics require rapid action, and therefore development of therapeutic treatments against the viruses of the respiratory tract is the need of the hour. Since the efficient diseased model for preclinical studies has not been developed so far, hence this is the biggest challenge in producing effective drugs against

these viruses. Currently, 2D in vitro cell cultures and animal models are being used as preclinical models that often result in failed association to human clinical trial responses. These methods are usually low throughput and do not mimic the function and physiology of the human body in its true sense. To fight the current COVID-19 pandemic, a team of researchers from Harvard University quickly leveraged the lung airway chip platform to test and repurpose the existing FDA-approved drugs as potent therapeutic molecules against SARS-CoV-2 (Tang et al. 2020).

The human airway chip is a microfluidic instrument consisting of two parallel microchannels, which are separated by a porous membrane coated with the extracellular matrix (Benam et al. 2016a, b). The air-liquid interface (ALI) of the chip is surrounded by primary human lung airway basal stem cells in the airway channel and primary human lung endothelium in the parallel vascular channel. This device leads to the differentiation of the lung airway basal stem cells. The stem cells differentiate into a mucociliary, pseudostratified epithelium along with other airway-specific cell types such as mucus-producing goblet cells, club cells, ciliated cells and basal cells. The organ chip also leads to the establishment of mucus production levels and permeability barrier properties comparable to the levels perceived in human airway in vivo (Yaghi and Dolovich 2016). The underlying human pulmonary microvascular endothelium also leads to a continuous planar cell monolayer where cells are interconnected by VE-cadherin containing adherens junctions as observed in vivo.

The differentiated epithelial cells in the airway chip are shown to express increased levels of various serine proteases such as TMPRSS2, TMPRSS4, TMPRSS11D and TMPRSS11E (DESC1) in comparison to MDCK cells that are generally tried to investigate influenza infection in vitro. These serine proteases are found to play an important role in the stimulation and spread of influenza and SARS-CoV-2 viruses in the human body. Moreover, differentiation of the airway basal stem cells at an ALI on-chip leads to overexpression of ACE-2 protein and mRNA of ACE-2 (angiotensin converting enzyme-2), which acts as a receptor for SARS-CoV-2 (Hoffmann et al. 2020).

Upon the infection of GFP-labelled H1N1 influenza virus into the air channel of the human airway chip to recapitulate the airborne influenza in vivo, it was observed through real-time fluorescence microscopic analysis that the virus has infected the airway epithelial cells. This resulted in damage to the epithelium, including absence of apical cilia, reduced barrier function as well as disruption of tight junctions. On the other hand, significantly less infection was observed in undifferentiated airway basal epithelium prior to being cultured on the airway chip. The lung endothelium also got damaged on the airway chip and was characterized by absence of VE-cadherin. The disruption was found to be consonant with the blood vessel leakage that is observed during an influenza infection in human lung in vivo (Benam et al. 2016a; Si et al. 2019, 2021; Armstrong et al. 2013). It was observed that the H3N2 virus strains were 10 times more replication efficient than the H1N1 virus strains and caused increased cilia loss and high barrier function damage in the microfluidic chip, which supports the finding that H3N2 causes more severe clinical symptoms and is more virulent and highly infectious (Cheung et al. 2002). When the

primary human neutrophils were perfused through the vascular channel of airway chips infected with H1N1 or H3N2, it was found that circulating immune cells were recruited to the apical surface of the activated lung endothelium within minutes. The neutrophils further transmigrated through the endothelium followed by the ECM-coated membrane, and finally reached into the airway epithelium in few hours (Si et al. 2021). The influenza nucleoprotein (NP)-expressing infected airway cells were targeted by neutrophils. Upon being targeted by neutrophils, the infected airway cells converged into clusters, and their size decreased over time, which resulted in clearance of the virus from the chip. More neutrophil recruitment was observed by H3N2 on the airway chip, which is concurrent with the observation that H3N2 virus leads to stronger and more dangerous inflammation as compared to H1N1 in vivo (Cheung et al. 2002). Oseltamivir (Tamiflu), the anti-influenza molecule used clinically, was tested to inspect if the airway chip could be used to analyse its efficacy. It was observed that Oseltamivir (1 μM) strongly repressed the replication of the influenza virus. It prevented disruption of epithelial tight junctions and defects of barrier function and diminished the synthesis of many cytokines and chemokines induced by the influenza virus on the airway chip. This implies that the airway chip truly repeats the effects of oseltamivir as observed in humans, indicating clearly that it can cater as an efficient 3D in vitro model to assess and understand the efficacy of potent anti-influenza molecules/drugs for virus-infected human lung disease as a part of preclinical studies (Si et al. 2021).

As microfluidic chip was faithfully able to recapitulate the human lung response to influenza, it was then studied as a model for SARS-CoV-2.

SARS-CoV-2 pseudo particles (SARS-CoV-2pp) were created to imitate human airway infection by airborne SARS-CoV-2. These pseudo particles carrying the SARS-CoV-2 spike protein (one of the main factors) were added into the air channel of the chip and were thus present in close proximity to the human lung epithelial cells expressing increased levels of TMPRSS2 and ACE2 (Tang et al. 2020; Armstrong et al. 2013). To explore the potential of using existing FDA-approved antiviral drugs, as COVID-19 preventive therapies, the CoV-2pp-infected human airway chips were treated by introducing drugs like *chloroquine*, *arbidol*, *toremifene*, *clomiphene*, *amodiaquine*, *verapamil*, or *amiodarone* into their vascular channel. Through these studies it was observed that only two drugs, i.e. *amodiaquine* and *toremiphen*, statistically repressed the viral infection (Si et al. 2021).

Another team of researchers from Massachusetts built a high-throughput human primary cell-based airway model to analyse and study respiratory viruses in vitro. The platform is known as PREDICT96- ALI and is based on the PREDICT96 organ-on-a-chip model, which consists of a microfluidic culture plate with 96 individual devices and a perfusion system driven by 192 microfluidic pumps integrated into the plate lid. The PREDICT96-ALI platform is observed to be effective as it is high throughput and summarizes healthy human airway structure and function. The model expresses ACE-2 and TMPRSS2 and also supports infection with human coronavirus. Thus, it can be considered as a potential cell-based airway model to study respiratory viruses in vitro (Gard et al. 2020).

In another study, a micro-engineered airway lung chip was modelled to study the viral induced exacerbation of asthma. A micro-engineered model of terminally differentiated human mucociliary airway epithelium was induced with IL-13 which stimulated a Th2-type asthmatic characteristic, and it was then infected with live human rhinovirus 16 (HRV16) to recapitulate the phenotypic features of asthma caused by virus (Nawroth et al. 2020).

17.3 Tissue Chips in Space

It has been well established that micro physiological systems or tissue chips serve as excellent models to study the diseased condition of the human organs and also for drug screening processes. It is observed that spaceflight leads to various changes in the body of the astronauts. Microgravity environment exerts numerous stresses and pathological and physiological disturbances on the human body leading to reduced cardiopulmonary function, osteoporosis, suppressed immune response and muscle wasting (Demontis et al. 2017; Tanaka et al. 2017; Fitzgerald 2017; Watenpaugh and Hargens 1996; Nicogossian et al. 2016). These changes directly correlate to aging and diseased pathology on Earth. The cellular and molecular mechanisms behind these changes remain unknown due to the limited availability of biosamples of the astronauts. Research works in mice as surrogate have been carried out that included transcriptomic, biochemical and proteomic analysis following a mission on the International Space Station by the mice (Hammond et al. 2018; Mao et al. 2018). However, whether these studies show that same human health effects and diseased conditions are produced as are produced in mice is still unknown. When a recent study was conducted on the jugular vein flow and morphology, it was first time found that obstructive thrombosis occurred in an astronaut in space (Aunon-Chancellor et al. 2020; Marshall-Goebel et al. 2019). Treatment was carried out using the available anticoagulants on board the International Space Station (ISS); although spontaneous flow was not found even after 3 months of treatment with apixaban on the ISS, it was surprisingly observed on landing (Aunon-Chancellor et al. 2020). The challenge is that the current knowledge of drug pharmacological kinetics and dynamics during spaceflight and return to Earth is uncertain (Eyal and Derendorf 2019), due to lack of research in the microgravity environment.

To overcome this challenge, a robust and accurate model system is required to mimic the physiology and functions of the human organs. Therefore, micro physiological systems or organ chips are being employed on board the ISS to identify the effects of microgravity on human bodies and to understand the mechanisms at the level of molecules, involved behind the changes that occur in a microgravity environment. The tissue chips research on board the ISS might give us an insight on two broad aspects; firstly, the physiological changes associated with microgravity are also associated with aging and diseased conditions on Earth. Therefore, tissue chips on board the ISS exhibit disease pathologies in expedited time frame which would take years on Earth. This research will help scientists to develop therapeutics against age-related diseases. Secondly, tissue chips serve as a model system for

changes in physiology and function of a human body during a spaceflight. This research will enable scientists to plan explorations deep into the space like future missions to the Moon and Mars at a reduced risk to health of the astronaut (Eyal and Derendorf 2020).

According to the "Tissue Chips in Space" move, by collaboration of the NCATS (National Center for Advancing Translational Sciences) and ISS, the following nine projects are being carried out at ISS. The goal of the Tissue Chips in Space initiative is to use tissue chip platforms and the exclusive microgravity environment of the ISS to develop models of human disease, with the ultimate goal of expediting the discovery of therapeutics for people on Earth (Low and Giulianotti 2019; Yeung et al. 2020).

A team from University of California, San Francisco (UCSF), is working on a project that investigates microgravity-induced aging of the immune system (generated through simulated microgravity and spaceflight) and its role in tissue-specific healing and regeneration using micro physiological systems (Low and Giulianotti 2019). It is known that spaceflight induces dysregulation of the immune system and inflammatory responses and thus can be considered as a surrogate model for Earth-based immunosenescence.

A team of researchers from MIT (Massachusetts Institute of Technology) is utilizing tissue chips to find therapeutics to treat post-traumatic osteoarthritis (PTOA). The tissue chip system comprised of human cartilage, bone and synovium that is challenged with inflammatory cytokines and an acute impact injury which is believed to be an appropriate model for PTOA. The model will be used to test therapeutic options and monitor their effectiveness through the use of intracellular and extracellular biomarkers (Low and Giulianotti 2019).

A study of proximal tubule proteinuria and distal tubule kidney stone formation is being conducted by a group from University of Washington (UW), leveraging the ISS environment. As disease progression of proteinuria, osteoporosis and kidney stone formation is often slow and difficult to model in vivo in terrestrial studies, the group is utilizing microgravity to accelerate the onset of diseased state using a human cell-derived kidney tissue chip system (Low and Giulianotti 2019; Yeung et al. 2020).

Another team from the UW, is using an engineered heart tissue chip to study aspects of cardiomyopathy related to human health on Earth and in space. The study is based on automated real-time continuous functional readouts of the engineered heart tissues using a novel magnetometer-based motion sensor. The ultimate goal is to test pharmaceuticals and mechanical stimulations as potential therapeutics (Low and Giulianotti 2019).

The first line of defence of a human body is the innate immune response which recruits innate immune cells to the infected organ or the site of action. A human airway tissue chip connected to a bone marrow tissue chip is being developed by the Children's hospital of Philadelphia (CHOP). By utilization of this interconnected tissue chip system, the team can infect the airway chip and keep track of the recruitment of neutrophils from the bone marrow as a model of innate response. The project is focused on the finding that spaceflight induces changes that involve

dysregulation of the immune system. This ISS project is expected to serve as a model for a compromised immune system (Low and Giulianotti 2019).

A team from the University of Florida (UF) aims to use the accelerated muscle wasting property of the microgravity by using a human muscle tissue chip as a model for sarcopenia in terrestrial settings. Sarcopenia is a disorder that usually affects the older adults and can be characterized as loss of skeletal muscle mass, similar to muscle wasting during spaceflight. The study will employ cells from different types of patients (human muscle cells isolated from young, healthy and older, sedentary volunteers) and will thus monitor the progression of the sarcopenia phenotype on a time scale not possible in terrestrial settings (Low and Giulianotti 2019).

Human induced pluripotent stem cells (iPSC) from healthy patients are being utilized by a team of researchers from Stanford University to fabricate engineered heart tissue platforms for use on the ISS and on the ground. By manipulating the microgravity-induced weakening of the heart muscle to their advantage, the team will validate the ISS platform as a tool to model ischemic cardiomyopathy in humans on Earth and later to screen for potential drug candidates to treat patients (Low and Giulianotti 2019).

A team of researchers from Emulate is developing an automated BBB (blood-brain barrier) tissue chip platform derived from human cells for use both on the ground and on the ISS. This model will allow the monitoring of the dysregulation of the BBB which could provide applications for studying neurological disorders as well as the transport of drugs and toxins to the central nervous system. Another team from Emulate will use plug-and-play technologies to modify their automated platform developed for the BBB ISS experiment to study dysregulation in the gut. This system will be infected to study the innate and probiotic-induced response (Low and Giulianotti 2019).

17.4 Micro Physiological Human Brain or Neural System on a Chip

Neurological diseases and disorders lead to majority of deaths worldwide (Feigin et al. 2020). Neurodegenerative diseases (NDDs) consist of hundreds of different types of neural system disorders in human beings in the globe. According to the World Health Organization (WHO), there is expected to be nearly 50% increase in the number of people suffering from NDDs by 2030 (Mofazzal Jahromi et al. 2019; Menken et al. 2000). Neurological disorders, diseases and injuries (NDDIs) are more common in the elderly and cause substantial degradation in the well-being of the patient.

Therapeutic treatments for various NDDIs have been developed over the years using 2D cell culture followed by animal models and subsequently clinical trials. But these are "reductionist approaches" that many a times fail to recapitulate the dynamic features, functionalities and trajectories of the in vivo nervous system (Pampaloni et al. 2007; Breslin and O'Driscoll 2013). Due to the many limitations of these preclinical model systems, "micro physiological nervous systems" (MPNS) come

into picture leading to advancement of the knowledge of the highly ordered structural features, role and disease of the human nervous system, which therefore would lead to the development of innovative medicines for NDDIs (Haring et al. 2017).

Chip-based MPNS are 3D culture systems based on a scaffold that replicate highly ordered structural aspects as well as roles of the nervous system. Design and engineering of a neural tissue chip is very challenging as it requires precise modelling of human neural circuitry, anatomy and microenvironmental conditions to achieve higher-order and dynamic pathophysiology or neurophysiology of the human body. Brain-on-a-chip or neural tissue chip is designed using a distinctive "structure-function-disease" logical perspective. Structural and functional components essential for handling system framework to attain higher performance levels are catered to (Haring et al. 2017). Hence, microfluidic brain-on-a-chip in conjugation with stem cells has great potential to be used as an effective research tool to study novel therapeutics for the treatment of various nervous system disorders.

Parkinson's disease (PD) is a complex neurodegenerative disorder observed by reduced number of neurons in the brain's basal ganglia. It affects 70% of the dopaminergic neurons of the *substantia nigra* of the midbrain, associated with onset of motor dysfunction (Davie 2008; Obeso et al. 2008). The in vitro models and animal models for the PD are not able to mimic the human pathology of PD and hence results in the failure of development of efficacious therapeutic molecules for PD in the clinical trials (Poewe et al. 2017; Meissner et al. 2011).

A group of researchers from the University of Luxembourg worked with 3D cultures for studying the high content phenotype and drug analysis of dopaminergic neurons involved in the development of pathology of PD (Bolognin et al. 2018). The same group has also shown that neuroepithelial stem cells derived from iPSCs can be differentiated into midbrain-specific dopaminergic neurons using 3D microfluidics instrument (Moreno et al. 2015). G2019S mutation in LRRK-2 (leucine-rich repeat kinase 2) commonly leads to PD. Mutated LRRK2 has been found to hinder various cellular pathways like cell proliferation, protein trafficking and cytoskeletal integrity (Healy et al. 2008; Wallings et al. 2015). 3D tissue chip culture showed that LRRK2-G2019S mutation leads to dopaminergic neuronal cell death. It was also observed that LRRK2-G2019S caused mitochondrial abnormalities and cell death in young neurons. When LRRK2 inhibitor (Inh2) was administered, it was found that it salvaged only few phenotypes. This indicates that apart from LRRK2-G2019S mutation, other genetic factors also contribute to phenotypes associated with PD. For this purpose, identification of these LRRK2-G2019S-dependent and independent phenotypes was done using high-content image analysis platform. It can be used to check the efficacy of potent therapeutic compounds in a high-throughput manner. Using iPSCs-derived human neuroepithelial stem cells on 3D tissue chips, they stated that in place of the LRRK2-G2019S mutation, it was the genotype of the patients that was found to be a major discerning factor among the lines (Bolognin et al. 2018). Matrigel as a scaffold was chosen as it contains high levels of extracellular matrix proteins such as laminin, collagen and heparin sulphate proteoglycans which also have been reported to be present in high levels in the brain extracellular matrix (Hughes et al. 2010). The 3D scaffold given by Matrigel and also other

hydrogels such as collagen and alginate gels has shown to expedite neuronal network formation on the chip (Choi et al. 2014; Ortinau et al. 2010).

According to another study using organ chips, it was shown that the vascular system leads to neuronal maturation of the spinal cord neural tissue. The signalling pathways have been found to be common in brain microvascular endothelial cells (BMECs) and neurons early in development, but their role in human neuronal maturation is mostly unknown. Scientists were able to develop both spinal motor neurons and BMECs from human induced pluripotent stem cells. The organ chips showed an elevated calcium transient function and gene expression in comparison to 96 well plates. Addition of BMECs in the organ chip led to specific gene expression and vascular-neural interaction that, in addition, augmented the neuronal function and in vivo-like physiology (Sances et al. 2018). It clearly shows that use of organ chips can direct stem cells cultured within them to move closer to an in vivo structure and functionality.

A recent study also stated the use of 3D brain-on-chip platform with human iPSC-derived brain cells as a model to evaluate the neurotoxicity of organophosphates (OP) exposure and to evaluate beneficial compounds for cure (Liu et al. 2020). OPs are nerve agents that inhibit acetylcholinesterase (AChE) and thus cause severe neurotoxicity (Eddleston et al. 2008). OPs are also observed to cause necrosis, apoptosis and alteration of oxidative stress-mediated pathway (Kashyap et al. 2011). Butyrylcholinesterase (BuChE) has been used as a cure for OP toxicity as BuChE and AChE have similar active sites and both efficiently catalyse the breakdown of acetylcholine (Ach). Inactivation of BuChE activity does not cause toxicity, as BuChE is not known to play an important role in vivo (Lee and Harpst 1971; Reiner et al. 2000; Masson and Lockridge 2010; Vijayaraghavan et al. 2013; Li et al. 2006; Broomfield et al. 1991; Lenz et al. 2005, 2010; Mumford et al. 2013). BuChE is a bioscavenger of OPs and does not allow them to reach their physiological targets (Nachon et al. 2013). A 3D brain-on-chip platform with human induced pluripotent stem cell (iPSC)-derived neurons and astrocytes to simulate human brain behaviour was developed. The chip consisted of two compartments: the first compartment contained a hydrogel embedded with human iPSC-derived GABAergic neurons and astrocytes, and the second compartment consisted of a perfusion channel with dynamic medium flow. These brain tissue constructs were treated with various concentrations of malathion (MT), an organophosphate insecticide which acts as an AChE inhibitor and subsequently exposed to BuChE after 20 min of MT treatment. Results depict that the iPSC-derived neurons and astrocytes came in close proximity of each other and developed synapses in the 3D matrix. Also the exposure to BuChE enhanced the viability after MT treatment (Liu et al. 2020). It was also observed that neurons require co-culturing with astrocytes for their growth, enhanced neuronal synaptic activity and heightened synaptic transmission (Johnson et al. 2007). A previous research conducted by the same team also showed the effects of astrocytes on viability using human iPSC-derived 3D brain-on-chip model (Liu et al. 2019). It was found that a higher number of astrocytes in comparison to neurons enhanced the viability following severe MT exposure which is also consistent with the previous studies indicating that astrocytes have been found to protect

against diazinon- and diazonxon-induced inhibition of neurite outgrowth (Pizzurro et al. 2014).

Opioid overdose leads to 69,000 deaths each year around the world, and 15 million people suffer from opioid addiction (Parthvi et al. 2019). Opioid abuse is a significant crisis to public health worldwide; therefore there is a desperate need to develop therapeutics for treatment of opioid use disorder (OUD) and also to develop pain treatments that are non-addictive. The addictive quality of opioid drugs involves variations in the activity of brain neurons that utilize dopamine as a neurotransmitter. The search for drugs that can reverse such changes and potentially treat addiction to opioid drugs, as well as the search for analgesics that are devoid of addictive qualities, will be aided by model systems based on human cells. A team of researchers from University of California, Los Angeles, is currently working to develop a multi-organ, micro physiological systems (MPSs) based on the use of human induced pluripotent stem cell (iPSC)-derived midbrain-fated dopamine (DA)/ GABA neurons on a three-dimensional platform that incorporates microglia, blood-brain barrier (BBB) and liver metabolism components. The model chip will focus on a key component of addictive circuitry—the dopaminergic and gamma-aminobutyric acid (GABA)ergic neurons of the midbrain, which have been recognized as responsible for mediating the reinforcing properties of many classes of abused drugs, including opioids (Ashammakhi et al. 2019).

The blood-brain barrier (BBB) is a highly selective semipermeable border of endothelial cells. It prevents the non-selective crossing solutes in the circulating blood into extracellular fluid of the central nervous system. The blood-brain barrier plays an important role in protecting and controlling entry of drugs to the brain tissue. The tight and stable BBB on the organ chip models has been difficult to maintain. According to a study by researchers from Harvard University, a tight blood-brain barrier can be maintained for a long period of time by regulating the amount of oxygen in an organ chip with stem cell-derived blood vessel cells (Park et al. 2019; van der Meer 2019). In a study carried out by researchers at Seoul National University in South Korea, the interactions were observed between glial cells and vascular cells, using the 3D micro vessel chip. It was observed that these interactions are important in tuning the geometry and function of brain microvascular networks. The extracellular matrix in which the cells are present has been found to control these interactions in part (van der Meer 2019; Lee et al. 2020).

Neural tissue chip platforms are also being used to observe the higher-order pathophysiology of Alzheimer's disease (AD). In a study at Korea University, it was found that when interstitial flow was applied to 3D neurospheroids on a microfluidic neural tissue chip, then Aβ was found to be significantly more damaging as compared to static conditions (Park et al. 2015).

17.5   A Few Other Recent Interesting Applications of the Organ-On-A-Chip Technology

17.5.1 Blood Vessels on a Chip

Graaf et al. used human induced pluripotent stem cells as a source of endothelial cells (hiPSC-ECs), along with a technique called viscous finger patterning (VFP) to develop in vitro cultures of human vascular cells inside microfluidic chips. In this finding, by providing the mechanical stimuli such as shear stress, they can emulate characteristics of the in vivo microenvironment. This is a robust model as it shows good results as far as the repeatability of the diameter between and within channels is concerned. All these features make hiPSC-EC-based MPS a cost-effective and efficient system for future studies in individualized disease modelling (de Graaf et al. 2019).

Duinen et al. studied a robust, high-throughput method to culture endothelial cells as 96 3D and perfusable micro vessels. They also fabricated a quantitative, real-time permeability assay to evaluate their barrier potential. As this assay has high-throughput value, high efficiency and robustness, hence it will be helpful for the researchers to make the transition from 2D to 3D culture models to observe and learn vasculature (van Duinen et al. 2017).

Poussin et al. have created a 3D model of endothelial micro vessels on a chip using primary human coronary arterial endothelial cells. This model will be used to study the attachment of monocyte to endothelium under flow and its applications in system toxicity (Poussin et al. 2020).

Duinen et al. developed a culture of perfusable 3D angiogenic sprouts in a membrane-free platform. A cocktail of angiogenic factors was added to the other side of the ECM gel, against which endothelial cells were grown on the chip. The resulting gradient of angiogenic factors induced the formation of endothelial sprouts through the ECM gel within 4 days. This perfused 3D angiogenesis model is amenable to high-throughput screening (van Duinen et al. 2019).

17.5.2 Tissue Chips as Disease Model for Oncological Studies

Lanz et al. analysed the use of a high-throughput organ-on-a-chip platform to select potent therapeutics for breast cancer. Triple negative breast cancer cell lines were seeded in the microfluidic platform. Cisplatin dose responses were generated in the simultaneous culture of 96 perfused micro tissues. The results have been promising and have created a possibility to the use of the organ-on-a-chip technology in individualized drug to ensure selection of efficient drugs and to determine the expected response to a particular treatment in real time (Lanz et al. 2017).

Aleman et al. described a metastasis-on-a-chip device that consisted of multiple bioengineered 3D organoids, made by a 3D photopatterning technique using extra-cellular matrix-derived hydrogel biomaterials. The cancer originated in colorectal cells chip and subsequently could be detected in other chips such as lung and liver

which are connected to the origin chip. This study is beneficial to gain knowledge of the mechanisms leading to metastasis, further in identification of anti-tumour drug targets (Aleman and Skardal 2019).

17.5.3 Organ-On-A-Chip Technology to Mimic the Fetomaternal Membrane Interface

Richardson et al. in 2019 created the first OOAC to mimic the components of foetal membranes to know the cell-cell interactions and paracrine crosstalk between maternal and foetal cells during pregnancy and parturition. The FM-OOC (fetomaternal organ-on-chip) platform used the vertical co-culture OOC design and was formed by two orthogonal vertically stacked cell culture chambers containing equal surface areas. The FM-OOC was used to analyse membrane permeability, oxidative stress and toxin-induced senescence, as well as cytokine production (Richardson et al. 2019a).

Richardson et al. in 2019 also created an amnion membrane OOC (AM-OOC), which was the first of its kind. This model successfully showed the interactive and transitional characteristics of amnion cells (epithelial-to-mesenchymal transition and mesenchymal-to-epithelial transition), under normal and oxidative stress conditions, similar to how they function and respond in utero (Richardson et al. 2019b).

Tissue chip has also been commercialized, and many companies such as Tissue, Emulate, Tara Biosystems, etc. have emerged from this technology. These companies develop specific tissue chips to meet the needs of their clients.

17.6   Discussion and Future Directions

The article has established that there are many exciting and innovative applications of the OOAC technology to study drug development and disease models. The tissue chip is a flexible model that can be utilized to study any diseased condition and pathology in the future. On the basis of the design and utility, they can be used to ask and answer the questions for which answers are not available by conventional means. Despite numerous advantages, there are still many tasks that need to be conquered for this technology to be considered as an ultimate model for drug screening.

Although OOAC has evolved and established quickly, the human on a chip theory remains far-off. There are disadvantages associated with the most commonly used material for production of tissue chips (i.e. PDMS). As the PDMS film is thicker than the in vivo morphology, hence this causes a decreased absorbance of small hydrophobic molecules which influence solvent efficacy and toxicity. Therefore, it is necessary to find suitable substitute materials. Another challenge in the use of this technology is that the cost of manufacturing is high and experimental use of organ chips is expensive and hence not suitable to the extensive usage of organ

chips. To overcome this challenge, the components of the organ chips must be cost-effective and easy to dispose.

But there is no doubt that tissue chip technology holds great future as a model to mimic disease pathology, hence proving to be beneficial for development of therapeutics for the rarest of the rare diseases.

In conclusion, microfluidic 3D models are very potent tools that will help the progressions in the field of biomedical research, leading to the development of novel therapeutics for the treatment of various human diseases.

References

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. https://doi.org/10.1002/bit.26871

Armstrong SM, Mubareka S, Lee WL (2013) The lung microvascular endothelium as a therapeutic target in severe influenza. Antiviral Res 99:113–118. https://doi.org/10.1016/j.antiviral.2013.05.003

Ashammakhi N, Khademhosseini A, Maidment NT, Seidlits SK, Svendsen CN (2019) Multi-organ-on-chip device for modeling opioid reinforcement and withdrawal, and the negative affective component of pain: a therapeutic screening tool. Project number: 1UG3TR003148-01. https://reporter.nih.gov/project-details/9883563#publications

Aunon-Chancellor SM, Pattarini JM, Moll S, Sargsyan A (2020) Venous thrombosis during spaceflight. N Engl J Med 382:89–90

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 (Basel) 11(6):599. Published 2020 Jun 18. https://doi.org/10.3390/mi11060599

Benam KH et al (2016a) Small airway on a chip enables analysis of human lung inflammation and therapeutic responses in vivo. Nat Methods 13:151–157. https://doi.org/10.1038/nmeth.3697

Benam KH et al (2016b) Matched-comparative modeling of normal and diseased human airway responses using a microengineered breathing lung chip. Cell Syst 3:456–466. https://doi.org/10.1016/j.cels.2016.10.003

Bhatia SN, Ingber DE (2014a) Microfluidic organs-on-chips. Nat Biotechnol 32(8):760–772. https://doi.org/10.1038/nbt.2989

Bhatia SN, Ingber DE (2014b) Microfluidic organs-on-chips. Nat Biotechnol 32(8):760–772. https://doi.org/10.1038/nbt.2989

Bolognin S, Fossépré M, Qing X et al (2018) 3D cultures of Parkinson's disease-specific dopaminergic neurons for high content phenotyping and drug testing. Adv Sci (Weinh) 6(1):1800927. Published 2018 Nov 20. https://doi.org/10.1002/advs.201800927

Breslin S, O'Driscoll L (2013) Three-dimensional cell culture: the missing link in drug discovery. Drug Discov Today 18:240–249. [PubMed: 23073387]

Broomfield CA, Maxwell D, Solana R, Castro C, Finger A, Lenz D (1991) Protection by butyrylcholinesterase against organophosphorus poisoning in nonhuman primates. J Pharmacol Exp Ther 259(2):633–638. PMID: 1941611

Chen L, Yang Y, Ueno H, Esch MB (2020) Body-in-a-cube: a microphysiological system for multi-tissue co-culture with near-physiological amounts of blood surrogate. Microphysiol Syst 4:1

Cheung CY et al (2002) Induction of proinflammatory cytokines in human macrophages by influenza a (H5N1) viruses: a mechanism for the unusual severity of human disease? Lancet 360:1831–1837. PMID: 12480361

Choi SH, Kim YH, Hebisch M et al (2014) A three-dimensional human neural cell culture model of Alzheimer's disease. Nature 515(7526):274–278. https://doi.org/10.1038/nature13800

Coller BS, Califf RM (2009) Traversing the valley of death: a guide to assessing prospects for translational success. Sci Transl Med 1(10):10cm9. https://doi.org/10.1126/scitranslmed.3000265

Davie CA (2008) A review of Parkinson's disease. Br Med Bull 86(1):109–127. https://doi.org/10.1093/bmb/ldn013

de Graaf MNS, Cochrane A, van den Hil FE et al (2019) Scalable microphysiological system to model three-dimensional blood vessels. APL Bioeng 3(2):026105. Published 2019 Jun 21. https://doi.org/10.1063/1.5090986

Demontis GC, Germani MM, Caiani EG, Barravecchia I, Passino C, Angeloni D (2017) Human pathophysiological adaptations to the space environment. Front Physiol 8:547

Eddleston M, Buckley NA, Eyer P, Dawson AH (2008) Management of acute organophosphorus pesticide poisoning. Lancet 371(9612):597–607

Eyal S, Derendorf H (2019) Medications in space: in search of a pharmacologist's guide to the galaxy. Pharm Res 36:148

Eyal S, Derendorf H (2020) One Giant leap for pharmacology. Pharm Res 37(3):62. https://doi.org/10.1007/s11095-020-02788-x

Feigin VL, Vos T, Nichols E et al (2020) The global burden of neurological disorders: translating evidence into policy. Lancet Neurol 19(3):255–265. https://doi.org/10.1016/S1474-4422(19)30411-9

Fitzgerald J (2017) Cartilage breakdown in microgravity-a problem for long-term spaceflight? NPJ Regen Med 11(2):10

Gard A, Maloney R, Cain B, Miller C, Luu R, Coppeta J, Liu P, Wang J, Azizgolshani H, Fezzie R, Balestrini J, Isenberg B, Finberg R, Borenstein J (2020) High-throughput human primary cell-based airway model for evaluating influenza, coronavirus, or other respiratory viruses in vitro. bioRxiv 2020.05.23.112797. https://doi.org/10.1101/2020.05.23.112797

Hammond TG, Allen PL, Birdsall HH (2018) Effects of space flight on mouse liver versus kidney: gene pathway analyses. Int J Mol Sci 19:4106

Haring AP, Sontheimer H, Johnson BN (2017) Microphysiological human brain and neural systems-on-a-chip: potential alternatives to small animal models and emerging platforms for drug discovery and personalized medicine. Stem Cell Rev Rep 13(3):381–406. https://doi.org/10.1007/s12015-017-9738-0

Healy DG, Falchi M, O'Sullivan SS et al (2008) Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol 7(7):583–590. https://doi.org/10.1016/S1474-4422(08)70117-0

Hoffmann M et al (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181(2):271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052

Hughes CS, Postovit LM, Lajoie GA (2010) Matrigel: a complex protein mixture required for optimal growth of cell culture. Proteomics 10(9):1886–1890. https://doi.org/10.1002/pmic.200900758

Huh D, Kim HJ, Fraser JP et al (2013) Microfabrication of human organs-on-chips. Nat Protoc 8(11):2135–2157. https://doi.org/10.1038/nprot.2013.137

Johnson MA, Weick JP, Pearce RA, Zhang S-C (2007) Functional neural development from human embryonic stem cells: accelerated synaptic activity via astrocyte coculture. J Neurosci 27(12):3069–3077. https://doi.org/10.1523/JNEUROSCI.4562-06.2007. PMID: 17376968

Kashyap MP, Singh AK, Kumar V, Tripathi VK, Srivastava RK, Agrawal M et al (2011) Monocrotophos induced apoptosis in PC12 cells: role of xenobiotic metabolizing cytochrome P450s. PLoS One 6(3):e17757

Kshitiz PJ, Kim P et al (2012) Control of stem cell fate and function by engineering physical microenvironments. Integr Biol (Camb) 4(9):1008–1018. https://doi.org/10.1039/c2ib20080e

Lanz HL, Saleh A, Kramer B et al (2017) Therapy response testing of breast cancer in a 3D high-throughput perfused microfluidic platform. BMC Cancer 17(1):709. Published 2017 Nov 2. https://doi.org/10.1186/s12885-017-3709-3

Lee JC, Harpst JA (1971) Purification and properties of butyrylcholinesterase from horse serum. Arch Biochem Biophys 145(1):55–63. https://doi.org/10.1016/0003-9861(71)90009-9. PMID: 5001230

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. https://doi.org/10.1002/bit.27224

Lenz DE, Maxwell DM, Koplovitz I, Clark CR, Capacio BR, Cerasoli DM et al (2005) Protection against soman or VX poisoning by human butyrylcholinesterase in Guinea pigs and cynomolgus monkeys. Chem Int 157:205–210. https://doi.org/10.1016/j.cbi.2005.10.025. PMID: 16289064

Lenz DE, Clarkson ED, Schulz SM, Cerasoli DM (2010) Butyrylcholinesterase as a therapeutic drug for protection against percutaneous VX. Chem Biol Interact 187(1–3):249–252. https://doi.org/10.1016/j.cbi.2010.05.014. PMID: 20513442

Li B, Duysen EG, Saunders TL, Lockridge O (2006) Production of the butyrylcholinesterase knockout mouse. J Mol Neurosci 30(1–2):193–195. PMID: 17192674. https://doi.org/10.1385/JMN:30:1:193

Liu L, Koo Y, Akwitti C, Russell T, Gay E, Laskowitz DT et al (2019) Three-dimensional (3D) brain microphysiological system for organophosphates and neurochemical agent toxicity screening. PLoS One 14(11):e0224657. https://doi.org/10.1371/journal.pone.0224657

Liu L, Koo Y, Russell T, Gay E, Li Y, Yun Y (2020) Three-dimensional brain-on-chip model using human iPSC-derived GABAergic neurons and astrocytes: Butyrylcholinesterase post-treatment for acute malathion exposure. PLoS One 15(3):e0230335. https://doi.org/10.1371/journal.pone.0230335

Low LA, Giulianotti MA (2019) Tissue chips in space: modeling human diseases in microgravity. Pharm Res 37(1):8. Published 2019 Dec 17. https://doi.org/10.1007/s11095-019-2742-0

Low LA, Tagle DA (2016) Tissue chips to aid drug development and modeling for rare diseases. Expert Opin Orphan Drugs 4(11):1113–1121. https://doi.org/10.1080/21678707.2016.1244479

Low LA, Tagle DA (2017) Tissue chips—innovative tools for drug development and disease modeling. Lab Chip 17(18):3026–3036. https://doi.org/10.1039/c7lc00462a

Mao XW et al (2018) Impact of spaceflight and artificial gravity on the mouse retina: biochemical and proteomic analysis. Int J Mol Sci 19:2546

Marshall-Goebel K, Laurie SS, Alferova IV, Arbeille P, Aunon- Chancellor SM, Ebert DJ et al (2019) Assessment of jugular venous blood flow stasis and thrombosis during spaceflight. JAMA Netw Open 2:e1915011

Marx U, Andersson TB, Bahinski A et al (2016) Biology-inspired microphysiological system approaches to solve the prediction dilemma of substance testing. ALTEX 33(3):272–321. https://doi.org/10.14573/altex.1603161

Masson P, Lockridge O (2010) Butyrylcholinesterase for protection from organophosphorus poisons: catalytic complexities and hysteretic behavior. Arch Biochem Biophys 494(2): 107–120. https://doi.org/10.1016/j.abb.2009.12.005. PMID: 20004171

Meissner WG, Frasier M, Gasser T et al (2011) Priorities in Parkinson's disease research. Nat Rev Drug Discov 10(5):377–393. https://doi.org/10.1038/nrd3430

Menken M, Munsat TL, Toole JF (2000) The global burden of disease study: implications for neurology. Arch Neurol 57:418–420. [PubMed: 10714674]

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

Mofazzal Jahromi MA, Abdoli A, Rahmanian M et al (2019) Microfluidic brain-on-a-chip: perspectives for mimicking neural system disorders. Mol Neurobiol 56(12):8489–8512. https://doi.org/10.1007/s12035-019-01653-2

Moreno EL, Hachi S, Hemmer K et al (2015) Differentiation of neuroepithelial stem cells into functional dopaminergic neurons in 3D microfluidic cell culture. Lab Chip 15(11):2419–2428. https://doi.org/10.1039/c5lc00180c

Mumford H, Docx CJ, Price ME, Green AC, Tattersall JE, Armstrong SJ (2013) Human plasma-derived BuChE as a stoichiometric bioscavenger for treatment of nerve agent poisoning. Chem Biol Interact 203(1):160–166. https://doi.org/10.1016/j.cbi.2012.08.018. PMID: 22981459

Nachon F, Brazzolotto X, Trovaslet M, Masson P (2013) Progress in the development of enzyme-based nerve agent bioscavengers. Chem Biol Interact 206(3):536–544. https://doi.org/10.1016/j.cbi.2013.06.012. PMID: 23811386

Nawroth JC, Lucchesi C, Cheng D et al (2020) A micro-engineered airway lung-chip models key features of viral-induced exacerbation of asthma. Am J Respir Cell Mol Biol 63(5):591–600. https://doi.org/10.1165/rcmb.2020-0010MA

Nguyen DH, Stapleton SC, Yang MT et al (2013) Biomimetic model to reconstitute angiogenic sprouting morphogenesis in vitro. Proc Natl Acad Sci U S A 110(17):6712–6717. https://doi.org/10.1073/pnas.1221526110

Nicogossian AE, Williams RS, Huntoon CL, Doarn CR, Polk JD, Schneider VS (2016) Space physiology and medicine. From evidence to practice. Springer-Verlag, New York

Obeso JA, Rodríguez-Oroz MC, Benitez-Temino B et al (2008) Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease. Mov Disord 23(Suppl 3):S548–S559. https://doi.org/10.1002/mds.22062

Ortinau S, Schmich J, Block S et al (2010) Effect of 3D-scaffold formation on differentiation and survival in human neural progenitor cells. Biomed Eng Online 9:70. Published 2010 Nov 11. https://doi.org/10.1186/1475-925X-9-70

Pampaloni F, Reynaud EG, Stelzer EH (2007) The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 8:839–845. [PubMed: 17684528]

Park J, Lee BK, Jeong GS, Hyun JK, Lee CJ, Lee SH (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(1):141–150. https://doi.org/10.1039/c4lc00962b

Park TE, Mustafaoglu N, Herland A et al (2019) Hypoxia-enhanced blood-brain barrier chip recapitulates human barrier function and shuttling of drugs and antibodies. Nat Commun 10(1):2621. Published 2019 Jun 13. https://doi.org/10.1038/s41467-019-10588-0

Parthvi R, Agrawal A, Khanijo S, Tsegaye A, Talwar A (2019) Acute opiate overdose: an update on management strategies in emergency department and critical care unit. Am J Ther 26(3):e387. https://doi.org/10.1097/MJT.0000000000000681

Pizzurro DM, Dao K, Costa LG (2014) Astrocytes protect against diazinon-and diazoxon-induced inhibition of neurite outgrowth by regulating neuronal glutathione. Toxicology 318:59–68. https://doi.org/10.1016/j.tox.2014.01.010. PMID: 24561003

Poewe W, Seppi K, Tanner CM et al (2017) Parkinson disease. Nat Rev Dis Primers 3:17013. Published 2017 Mar 23. https://doi.org/10.1038/nrdp.2017.13

Poussin C, Kramer B, Lanz HL et al (2020) 3D human microvessel-on-a-chip model for studying monocyte-to-endothelium adhesion under flow—application in systems toxicology. ALTEX 37(1):47–63. https://doi.org/10.14573/altex.1811301

Ramadan Q, Zourob M (2020) Organ-on-a-chip engineering: toward bridging the gap between lab and industry. Biomicrofluidics 14(4):041501. Published 2020 Jul 14. https://doi.org/10.1063/5.0011583

Reiner E, Sinko G, Skrinjaric-Spoljar M, Simeon-Rudolf V (2000) Comparison of protocols for measuring activities of human blood cholinesterases by the Ellman method. Arhiv za higijenu rada i toksikologiju 51(1):13–18. PMID: 11059068

Richardson L, Gnecco J, Ding T et al (2019a) Fetal membrane organ-on-chip: an innovative approach to study cellular interactions [published online ahead of print, 2019 Feb 21]. Reprod Sci:1933719119828084. https://doi.org/10.1177/1933719119828084

Richardson L, Jeong S, Kim S, Han A, Menon R (2019b) Amnion membrane organ-on-chip: an innovative approach to study cellular interactions. FASEB J 33(8):8945–8960. https://doi.org/10.1096/fj.201900020RR

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

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(4):1222–1236. https://doi.org/10.1016/j.stemcr.2018.02.012

Sato T, Clevers H (2013) Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. Science 340(6137):1190–1194. https://doi.org/10.1126/science.1234852

Scannell JW, Blanckley A, Boldon H, Warrington B (2012) Diagnosing the decline in pharmaceutical R&D efficiency. Nat Rev Drug Discov 11(3):191–200. Published 2012 Mar 1. https://doi.org/10.1038/nrd3681

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(40):12516–12521. https://doi.org/10.1073/pnas.1516645112

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. Published 2015 Nov 12. https://doi.org/10.1063/1.4935594

Si L et al (2019) Discovery of influenza drug resistance mutations and host therapeutic targets using a human airway chip. bioRxiv 685552

Si L, Bai H, Oh CY, Jin L, Prantil-Baun R, Ingber DE (2021) Clinically relevant influenza virus evolution reconstituted in a human lung airway-on-a-chip. Microbiol Spectr 9(2):e0025721. https://doi.org/10.1128/Spectrum.00257-21

Song JW, Daubriac J, Tse JM, Bazou D, Munn LL (2012) RhoA mediates flow-induced endothelial sprouting in a 3-D tissue analogue of angiogenesis. Lab Chip 12(23):5000–5006. https://doi.org/10.1039/c2lc40389g

Tanaka K, Nishimura N, Kawai Y (2017) Adaptation to microgravity, deconditioning, and countermeasures. J Physiol Sci 67(2):271–281

Tang H et al (2020) Human organs-on-chips for virology. Trends Microbiol 28(11):934–946. https://doi.org/10.1016/j.tim.2020.06.005

Theobald J, Ghanem A, Wallisch P, Banaeiyan AA, Andradenavarro MA, Taskova K, Haltmeier M, Kurtz A, Becker H, Reuter S (2018) Liver-kidney-on-chip to study toxicity of drug metabolites. ACS Biomater Sci Eng 4(1):78–89

Tibbe MP, Leferink AM, van den Berg A, Eijkel JCT, Segerink LI (2018) Microfluidic gel patterning method by use of a temporary membrane for organ-on-chip applications. Adv Mater Technol 3:1700200. https://doi.org/10.1002/admt.201700200

Tufts CSDD (2014) Cost to developing and win marketing approval for a new drug in $2.6 billion. Center for the Study of Drug Development. Published 2014 Nov 18

van der Meer AD (2019) Organs-on-chips: latest developments. Microphysiol Syst 3:1. https://doi.org/10.21037/mps.2018.12.01

van Duinen V, van den Heuvel A, Trietsch SJ et al (2017) 96 perfusable blood vessels to study vascular permeability in vitro. Sci Rep 7(1):18071. Published 2017 Dec 22. https://doi.org/10.1038/s41598-017-14716-y

van Duinen V, Zhu D, Ramakers C, van Zonneveld AJ, Vulto P, Hankemeier T (2019) Perfused 3D angiogenic sprouting in a high-throughput in vitro platform. Angiogenesis 22(1):157–165. https://doi.org/10.1007/s10456-018-9647-0

Vijayaraghavan S, Karami A, Aeinehband S, Behbahani H, Grandien A, Nilsson B et al (2013) Regulated extracellular choline acetyltransferase activity—the plausible missing link of the distant action of acetylcholine in the cholinergic anti-inflammatory pathway. PLoS One 8(6):e65936. https://doi.org/10.1371/journal.pone.0065936

Wallings R, Manzoni C, Bandopadhyay R (2015) Cellular processes associated with LRRK2 function and dysfunction. FEBS J 282(15):2806–2826. https://doi.org/10.1111/febs.13305

Watenpaugh DE, Hargens AR (1996) The cardiovascular system in microgravity. In: Fregly MJ, Blatteis CM (eds) Handbook of physiology, the gravitational environment. American Physiological Society, Rockville, MD

Wu Q, Liu J, Wang X et al (2020) Organ-on-a-chip: recent breakthroughs and future prospects. Biomed Eng Online 19(1):9. Published 2020 Feb 12. https://doi.org/10.1186/s12938-020-0752-0

Xue D, Wang Y, Zhang J, Mei D, Wang Y, Chen S (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

Yaghi A, Dolovich MB (2016) Airway epithelial cell cilia and obstructive lung disease. Cell 5:40. https://doi.org/10.3390/cells5040040

Yang K-S, Cheng Y-C, Jeng M-S, Chien K-H, Shyu J-C (2014) An experimental investigation of micro pulsating heat pipes. Micromachines 5:385–395

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(1):48–56. https://doi.org/10.1016/j.yexcr.2017.03.026

Yeung CK, Koenig P, Countryman S, Thummel KE, Himmelfarb J, Kelly EJ (2020) Tissue chips in space-challenges and opportunities. Clin Transl Sci 13(1):8–10. https://doi.org/10.1111/cts.12689

Zhou J, Khodakov DA, Ellis AV, Voelcker NH (2012) Surface modification for PDMS-based microfluidic devices. Electrophoresis 33(1):89–104. https://doi.org/10.1002/elps.201100482