Chapter 4
Organ-on-a-chip Systems

Aleksandra Szuplewska, Michal Chudy and Zbigniew Brzozka

4.1 From Monolayer Cultures to *Organ-on-a-chip* Systems—An Introduction

Current laboratory preclinical trials for novel drugs are based on a combination of in vitro cell culturing protocols and in vivo animal models (Esch et al. 2014a; Selimovic et al. 2013). Despite the high utility of a conventional cellular monolayer (two-dimensional, 2D) model in biomedical applications, the absence of specific tissue–tissue (and organ–organ) interaction and the lack of many cell type functions preclude anticipation of biological activity (e.g., possible mechanisms of action, potential toxic effects, impact on target-free tissues) (Esch et al. 2014b; Greek and Menache 2013; Jastrzebska et al. 2015). Therefore, three-dimensional (3D) models, mainly based on hydrogel technology, are highly valued in investigations on signaling pathways (see Chap. 3). 3D models more precisely mimic the chemical and spatial complexity of living tissues in comparison to 2D models (DiMasi and Grabowski 2007; DiMasi et al. 2010; Esch et al. 2014b). Organoids, i.e., self-organizing cellular microstructures are one of the spatial models. Although self-assembling tissues represent human organ physiology and functions more realistically, one of most meaningful organoid drawbacks is a strong correlation between their behavior and specific microarchitecture (size, shape, etc.). Another example of 3D model limitation is the suppression of further genetic, biochemical, and functional (e.g., investigation on secretion, transport, or interface phenomena) analysis by the difficulties in sample preparation (Ingber 2003; Mammoto et al. 2013). Moreover, the lack of fluid flow and other stimuli such as mechanical stretching or shear fluid stress in static 3D models is the next obstacle. In addition, the critical issue for the functioning of almost every organ is the interface between

A. Szuplewska (✉) · M. Chudy · Z. Brzozka
The Chair of Medical Biotechnology, Faculty of Chemistry,
Warsaw University of Technology, Warsaw, Poland
e-mail: aszuplewska@ch.pw.edu.pl

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Z. Brzozka and E. Jastrzebska (eds.), *Cardiac Cell Culture Technologies*,
https://doi.org/10.1007/978-3-319-70685-6_4

neighboring tissues, which is lacking in many systems based on conventional 3D models (Huh et al. 2011; Park and Shuler 2003; Sung et al. 2013; Ye et al. 2013). Animal models, regardless of ethical questions and difficulties in the proper interpretation of the obtained results, are not representative of most human organ functions, diseases, and responses to medical treatment (Esch et al. 2014b).

An alternative solution, potentially overcoming many of the limitations listed above, is novel platforms, known as *Organ-on-a-chip* systems (Bhise et al. 2014; Bhatia and Ingber 2014; Tomecka et al. 2018; Wang et al. 2016; Zuchowska et al. 2017). The connection of bio-microelectromechanical systems (bioMEMS) (Bashir 2004), biomimetics (Bhushan 2009), and microfluidics (Whitesides 2006) more appropriately mimics the in vivo microenvironment, e.g., simulation of activity, mechanisms and physiological responses on the tissue- and organ-level, in comparison to static 2D and 3D cellular models, as well as compared to cell culturing with the use of conventional microfluidic devices. It should also be noted that *Organ-on-a-chip* systems are currently the tools, which represent the minimal functional units—answering the physiological functions and responses of organs and tissues—rather than the whole living organ. Scheme of the *Organ-on-a-chip* concept is shown in Fig. 4.1. The simplest variant of *Organ-on-a-chip* systems includes analysis of one type of tissue/organ, but *Organ-on-a-chip* technology also offers the possibility of creating more complex, multi-organ platforms known as *Body-on-a-chip* or *Human-on-a-chip* (Beebe et al. 2013; Bhatia and Ingber 2014; Esch et al. 2015; Huh et al. 2013; Moraes et al. 2012; Moyer 2011; Polini et al. 2014; Shuler and Esch 2010; Sung et al. 2014; Wang et al. 2016). Such an integration allows the research on inter-tissue and interorgan communication as well as the simulation of human metabolism which plays a key role in studies on toxic and dose-related effects of novel therapies. Moreover, *Body-on-a-chip* systems allow for the integration of human induced pluripotent stem cells (hiPSC) to create connections between the patient and specific organ, which could potentially be developed for personalized medicine (Grskovic et al. 2011). There are various organs which have been mimicked in the microsystems: e.g., lung, liver, spleen, kidney, brain, and heart. To show a wide range and potential of this research, examples of *Organ-* and *Body-on-a-chip* systems are reviewed and discussed in this chapter. Cardiac cell technologies performed in microscale are presented in the following Chaps. 7–9.

4.2 *Organ-on-a-chip* Systems: Mimicking Physiological and Pathophysiological Conditions

The great limitation of classical models, based on 2D and 3D cell cultures, was the lack of physiological accuracy and inability to mimic the microenvironment on specific organ or tissue level. Contrary, the devices based on the *Organ-on-a-chip* concept which mimics conditions in human organs have great potential in studies

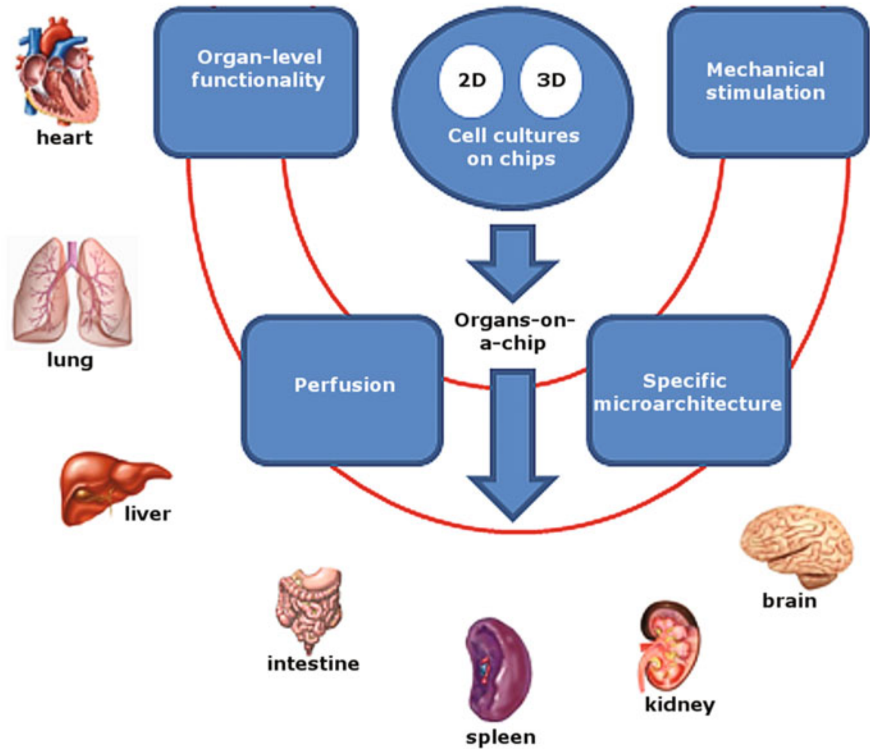

Fig. 4.1 *Organ-on-a-chip* concept

on biological phenomena by the presence of fluid flow, shear stress, and specific microarchitecture (Huh et al. 2011; Jastrzebska et al. 2016). Organs such as e.g., lung, liver, spleen, kidney, brain, and heart can be mimicked in the microsystems. It should be noted that cells coming from different organs have precisely defined properties. Therefore, to obtain the microsystems for simulation of a defined organ, specific features and parameters should also be fulfilled. Features such as dynamic fluid flow, mechanical stimulation, or material composition are taken into consideration during the fabrication of *Organ-on-a-chip* systems.

4.2.1 Mimicking of Organ Functions—The Examples

One of the key issues in the development of complex living systems is the ability to control and mimic biological 3D microarchitecture resulting from the creation of spatiotemporal, chemical microenvironments as well as the variety of used modules. The reconstruction of specific tissue functions and precise control of dynamic fluid flow has been often demonstrated (Folch and Toner 2000; Khademhosseini

et al. 2006; Mrksich et al. 1997; Whitesides et al. 2001; Whitesides 2006). Recently reported 3D models combine the reconstitution of complex tissue- and organ-level microarchitecture with mechanical as well as chemical and electrical stimulus. To achieve more accurate in vivo microarchitecture mimicking specific organs, the device based on 3D model with membrane-based multilayer compartments concept has been proposed. Such systems can be particularly useful in studies on biological barriers, as e.g., blood–brain barrier, alveolar–capillary interface in lungs, the gastrointestinal tract, the transport barrier in kidneys as well as the interface between the tumor and its external microenvironment. Some organs are simultaneously exposed to mechanical stimulation such as peristaltic motions, shear stress, or muscle contractions. Therefore, such features are also mimicked in the microsystems. Additionally, spatial arrangement of the cells (aggregates, hydrogels, nanofibers) is created inside the microsystems for more physiological accuracy of the designed systems. For example, the cerebral cortex in the brain (Alcendor et al. 2013), tumor aggregates of tumor tissue (Kim et al. 2012a; Lee et al. 2014; Mehta et al. 2012), bone marrow hematopoietic niches (Torisawa et al. 2014), or functional units of liver sinusoids (Lee et al. 2007; Schutte et al. 2011, 2009) were described as 3D models with the microenvironment regulation. To underline a wide range of the microsystem utilization for organ mimicking, we present examples of various *Organ-on-a-chip* systems in this section. Specific features, which should be fulfilled by the microsystems for simulation of specific organ, are also presented here.

4.2.1.1 Intestine-on-a-chip

A human intestine model is highly appropriable for investigation on the processes of transport, absorption, and metabolism of pharmaceutical and nutritious substances (Giacomini et al. 2010; Hodgson 2001). To mimic the specific microarchitecture, density and functionality of human intestinal villi, the microfluidic platforms for hydrogel-based spatial culture of intestinal epithelial cells are often utilized. Assembling the epithelial barrier is enabled by the use of a Transwell filter, one of the most commonly utilized conventional in vitro models of a human gut. Simultaneous reconstruction of mechanical and structural intestine properties under pathophysiological conditions along with a specific microbial flora is the key issue for biomedical applications (e.g., drug development) (Ferrec et al. 2001; Mahler et al. 2009). A porous membrane was used in a human *Gut-on-a-chip* system for investigating most meaningful intestine functions under physiological conditions, e.g., fluid flow, gut microbial flora, and cyclic mechanical strain (Huh et al. 2013; Kim et al. 2013). The designed microdevice consisted of two fluidic microchannels separated by a porous, highly flexible membrane coated with an extracellular matrix (ECM) and lined with cells derived from human intestinal epithelium (Fig. 4.2). Artificial peristaltic motions were generated on the cellular monolayer by full height vacuum chambers which caused mechanical strain (Kim et al. 2012b). The proposed model enabled observation of increase of intestinal barrier functionality.

Epithelial cell differentiation and formation of spatial villus-like aggregates were obtained thanks to the usage of low fluid flow rate (30 μL h^{-1}) and shear stress present in the living intestine (0.02 dyne cm^{-2}). The obtained results showed that the analyzed responses of the cells could potentially be enhanced by cyclic mechanical strain used in this model, in comparison to conventional cultures based on Transwell filters.

Among available in vitro human gut models, the most common solutions include the formation of polarized epithelial monolayers from established epithelial cell lines. A microsystem, which allows for forming intestinal villi and inducing Caco-2 cell spontaneous robust morphogenesis, was described by Huh et al. (2013). Analogously to normal human small intestine, basal cells continuously populate the

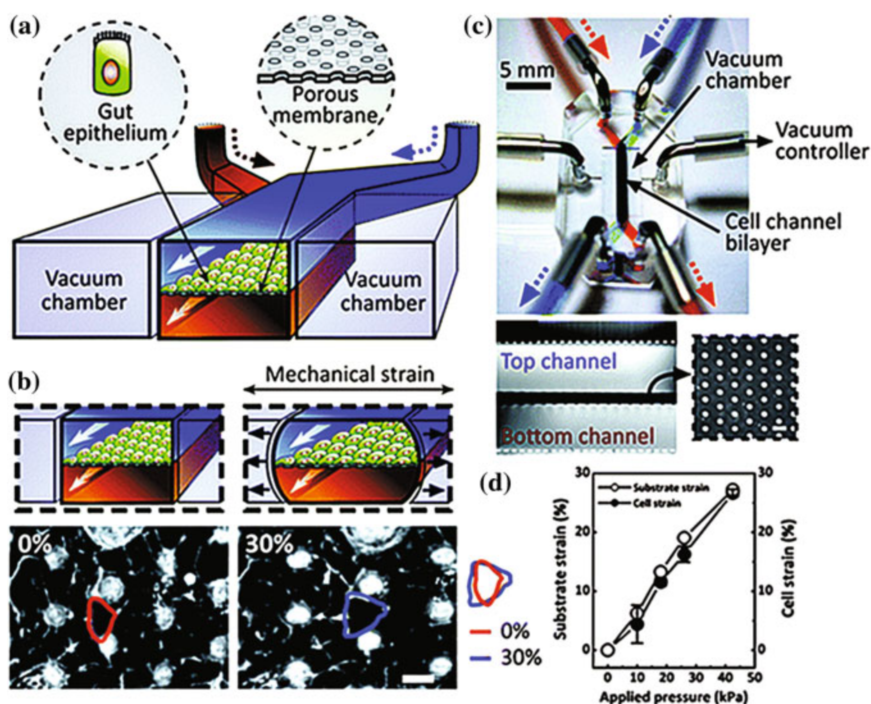

Fig. 4.2 **a** A scheme of the *Gut-on-a-chip* device with the flexible porous extracellular matrix (ECM)-coated membrane lined by gut epithelial cells crosses horizontally through the middle of the central microchannel, and full height vacuum chambers on both sides. **b** A scheme and phase contrast images of intestinal monolayers cultured within the *Gut-on-a-chip* in the absence (left) or presence (right) of mechanical strain (30%; arrow indicated direction) exerted by applying suction to the vacuum chambers. Red and blue outlines indicate the shape of a single Caco-2 cell before (red) and after (blue) mechanical strain application. **c** A photographic image of the *Gut-on-a-chip* device composed of PDMS elastomer and a cross-sectional view of the top and bottom channels (both 150 μm high) of the microsystem. **d** The graph showing dependence pressure on substrate strain. Reprinted with permission from Kim et al. (2012b). Copyright 2012 Royal Society of Chemistry

villi along the crypt–villus axis, and differentiate into four types of epithelial cells (mucussecretory, enteroendocrine, absorptive, and Paneth cell types). The intestinal surface and efficiency of absorption processes comparable to the normal human gut were obtained in the proposed model. Moreover, an enhancement of cytochrome P450 3A4 isoform-based drug metabolizing activity was observed compared with static device with Transwell membrane.

4.2.1.2 Lung-on-a-chip

Pulmonary diseases are currently one of the major causes of death worldwide. The number of these cases has been increasing in recent decades. Therefore, attentions have recently focused on some novel strategies with the aid of cell culturing models and microfluidic techniques not only for functional analysis, but also for drug screening (Doryab et al. 2016). The smallest elementary unit, distinguishable in lung tissue, is made of an endothelial and epithelial cell layer (van der Meer and van den Berg 2012). The high structural and geometric complexity, precluding the direct analysis of cells located in different regions of the lung (e.g., luminal surface coated by epithelium) as well as the mechanics of the organ and the presence of a large variety of physical stimuli, make a lung one of the most challenging organs to research under in vivo-like conditions.

Mechanical stimulation, e.g., cell exposition to fluid and continuous stress, is essential for mimicking of differences in alveoli microenvironment (Tavana et al. 2011). A strong correlation between the collapsing and reopening of alveoli causing cyclic and fluidic mechanical stresses was investigated. In acute respiratory distress syndrome, mechanical ventilation is the most commonly used form of therapy, while its use might induce further ventilator lung injury with exacerbation of acute respiratory distress syndrome (ARDS)-like symptoms (Douville et al. 2011). To mimic the cyclic stretch and fluid mechanical stress conditions, a microfluidic alveolar system was designed (Fig. 4.3). It consisted of an “alveolar chamber” and “actuation channel” which are separated by a membrane made of poly(dimethyl siloxane) (PDMS) (Douville et al. 2011).

During the process of gas exchange, air is transported between the external environments by small airways. Simultaneously, the gas exchanged in alveoli is transported via the lung barrier between blood and air (Huh et al. 2007). The alveoli gas exchange process might be impeded as a consequence of the formation of liquid plugs, a highly viscous film blocking the small airway epithelium. The observed effect might be induced by airway lumen air–liquid two-phase instabilities and accompany numerous pulmonary dysfunctions (Jacob and Gaver 2005). Respiratory crackles, i.e., a pathological sound induced by plug rupture during the process of occluded airways reopening, are one of the most widely used indicators of respiratory diseases in clinical practice (Grotberg 2001). An air–liquid two-phase microfluidic platform was proposed as the tool for studying the possible consequences of liquid plug propagation and rupture (Huh et al. 2007). The chip allow to mimic the pathophysiological conditions in respiratory system. The designed chip

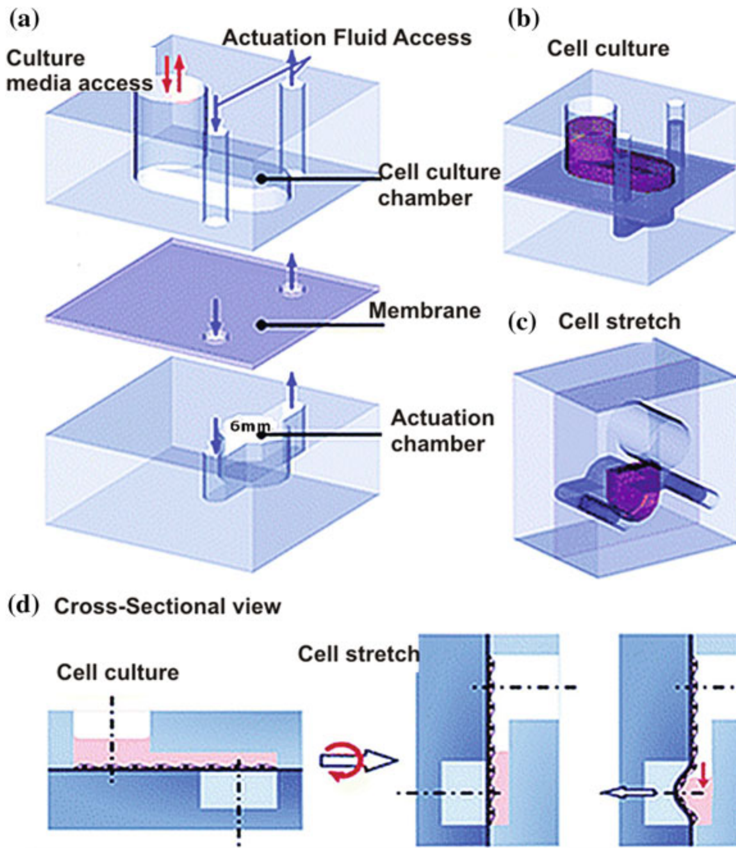

Fig. 4.3 **a** A scheme of *Alveoli-on-a-chip* system. A thin PDMS membrane separates the alveolar chamber for cell culture from the actuation channel used for deformation of the cell culture chamber. **b** The "alveolar chamber" can be filled with F-12K growth media and positioned in the horizontal orientation to allow for optimal cell adherence and growth. **c** During experiments, the "alveolar chamber" can be partially filled with fluid and positioned in the vertical configuration to establish a meniscus at the interface of fluid and air. **d** A cross-sectional view of the microsystem shows the horizontal orientation for cell culture and the vertical configuration for experimentation. Scheme of membrane deforming and cell stimulation. Reprinted with permission from Douville et al. (2011). Copyright 2010 Royal Society of Chemistry

was composed of a polymeric membrane (pore size: 400 nm), separating two chambers made of PDMS. The lower chamber was related to basal compartments of the airway epithelial layer while the upper one to the airway lumen. Working principle and design of a *Lung-on-a-chip* system presented by Stucki et al. (2015) is shown in Fig. 4.4.

The reconstruction of the main mechanical, functional, and structural characteristics of interface between alveoli and capillaries is allowed by the use of highly

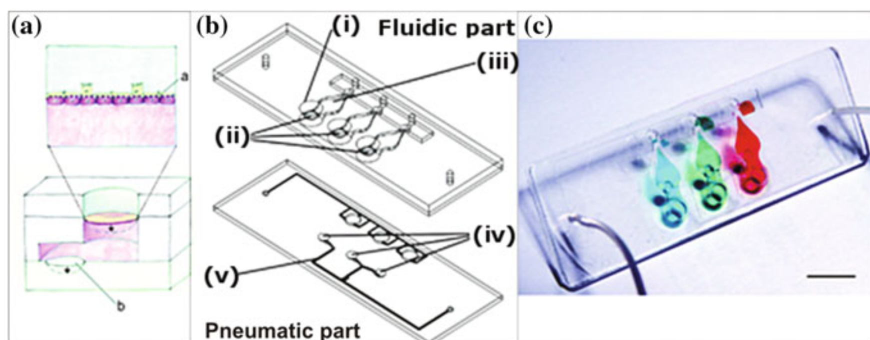

Fig. 4.4 Working principle and design of the *Lung-on-a-chip*. **a** In vitro, the 3D cyclic mechanical strain of the bioartificial alveolar membrane (a) induced by a micro-diaphragm (b) that is actuated by an electro-pneumatic setup. The bioartificial alveolar membrane consists of a thin, porous, and stretchable membrane on which epithelial and endothelial cells are cultured. **b** A scheme of *Lung-on-a-chip* system, which consists of three alveolar cell culture wells (i) and thin, porous, and flexible membranes (ii), beneath which the basolateral chambers are located (iii). The micro-diaphragms (iv) are integrated into the pneumatic part and connected to pneumatic microchannels (v). **c** Photograph of the microsystem filled with food-dye-colored solutions inside the basolateral chambers. Reprinted with permission from Stucki et al. (2015). Copyright 2015 Royal Society of Chemistry

flexible and permeable membranes. Track-etched polycarbonate (PC), as an example of a material which is quite stiff and permeable, does not accurately represent a mechanically dynamic environment. Otherwise, highly flexible, casted membranes, like those made of PDMS, act as a sealant to provide breathing mechanical movements. To create physiologically accurate alveoli, Huh et al. (2010) designed a platform based on two compartments separated by a porous, flexible membrane made of PDMS, coated with fibronectin or collagen (proteins naturally present in ECM). Mimicry of alveolar air space was made possible by introducing air into the upper compartment, where the human cells of alveolar epithelium were cultured. The human pulmonary endothelial cells were seeded on the opposite side of the intervening membrane. The structure of the chip consists of two lateral, larger microchambers for mimicry of respiratory movements, causing dynamic mechanical distortion of the alveolar–capillary interface. It was observed that the attached PDMS membrane and the adherent tissue layers stretch. Investigations on organ-level responses to cytokines inflammatory and microbial contamination were performed using the designed chip. The described system was also a useful tool for predicting potential nanotoxic effects—the obtained results showed that the cyclic mechanical strain impacts the cytotoxicity of silica nanoparticles. The reconstruction of inter-tissue borders and mechanical stimulation conditions—essential for lung functionality—makes the device reported on a suitable tool for predicting potential toxic and therapeutic effects.

4.2.1.3 Kidney-on-a-chip

One of the most meaningful issues of novel biologically active substances and alternative therapies development is potential nephrotoxic effects, often involved in a variety of processes such as active clearance, reabsorption, and local interstitial drug accumulation (Perazella 2009; Schetz et al. 2005; Tiong et al. 2014). Nephron is the minimal functional unit of kidney. Three main components such as the *glomerulus*, proximal convoluted tubule, and loop of Henle are distinguished in nephron (Weinberg et al. 2008).

The earlier reported research on the drug-related effects of tubular injuries was performed with the use of tissue slices, isolated cells, and commercially available kidney cell lines cultured on the membranes of Transwell insert dishes—porous or fabricated of polymeric materials (Baudoin et al. 2007; Gunness et al. 2010; Zhou et al. 2014). The proposed cellular kidney models based on the biology of phenotypically homogenous cells do not represent physiologically relevant phenotypic characteristics of primary renal proximal convoluted tubule cells. As a consequence of constant flow through the glomerular filtrate, the proximal tubule epithelium on both the basal and apical surfaces is stimulated under shear stress conditions. Because of the basal surface exposition to shear stress, the active transport of a number of biologically important components such as amino acids, drug metabolites, and glucose through epithelium is allowed (Jang et al. 2013). For better understanding of shear stress-related phenomena and renal functionality, a number of microfluidic platforms replacing the conventional and static Transwell culturing systems have been demonstrated (Duan et al. 2008, 2010; Jang et al. 2013). To create a basal "interstitial" space as well as an apical "luminal" channel, Jang et al. (2011) designed a microfluidic platform for culture of primary kidney epithelial cells. The microsystems consisted of one main culturing chamber separated into two adjacent channels by a porous, polyester membrane, which was coated with components present in ECM (Fig. 4.5). The formation of specific microarchitectures as well as apical fluid shear stress exposition was attained by cell culturing in the upper side of used membrane. The observed effect of attaching the epithelial cells to the cellular monolayer and enhanced by cell polarization has a significant impact on glucose reabsorption and transport of albumins. In comparison to conventional, static Transwell culturing platforms, the differences in alkaline phosphatase activity of the brush border epithelial cells were also observed. The utility for preclinical renal toxicity trials of the use of the in vivo-like kidney proximal *Tubule-on-a-chip* systems has been proven. In the microsystem, the rearrangement of the cytoskeleton, enhanced cell polarization, and reinforced cell junctions under exposition to hormonal stimuli was tested. The major role of inner medullary collecting duct (IMCD) cells in processes of water regulation and ion balance by molecular transport as the effect of hormonal stimulation in in vivo-like tubular environments was also developed.

As was mentioned before, nephron is characterized by three main components: the *glomerulus*, proximal convoluted tubule, and loop of Henle. Therefore to obtain good in vivo-like model, all nephron functionality should be mimicked. It should be

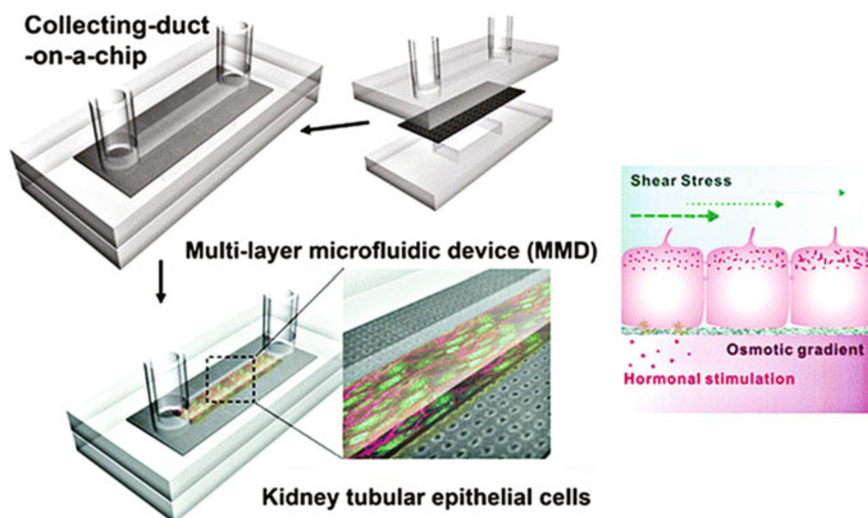

Fig. 4.5 A scheme of *Kidney-on-a-chip* system in which a flow and transport barrier functions were simulated. The microsystems consisted of one main culturing chamber separated into two adjacent channels by a porous, polyester membrane, on which kidney epithelial cells were cultured. A continuous hydrodynamic flow that produces shear stresses was also applied. Reprinted with permission from Jang et al. (2011). Copyright 2010 Royal Society of Chemistry

noted that a microfluidic system to mimic nephron features e.g., filtration of the glomerular unit with the filtration fraction was proposed by Weinberg et al. (2008). The microsystem consisted of a porous membrane, which played the role of artificial *glomerulus* and separated two microchip layers. In this part of the designed system, some components of a circulating blood sample might pass through the porous membrane into the filtrate as part of the formation of urine. Then, the filtrate flows into the proximal convoluted tubule section, where the processes of active reabsorption through the membrane take place. The reabsorption of ions and remaining water molecules takes place in the looping channels, mimicking the countercurrent mechanism of the loop of Henle. Nephron functions such as osmosis, diffusion, and active pumping are accurately reconstructed in the proposed microsystem. Thanks to that the reabsorption of remaining filtrate fluid has a final output of highly concentrated waste urine (the final concentration: 200–400 $\times$ 10^{-3} mol dm^{-3}).

4.2.1.4 Spleen-on-a-chip

The spleen is a lymphoid organ, which selectively filters infected, senescent, and damaged red blood cells as well as parasites from the *Plasmodium* family which are responsible for infections originating in the blood (Bowdler 2001). The blood surveillance function is connected with specific spleen microarchitecture, which

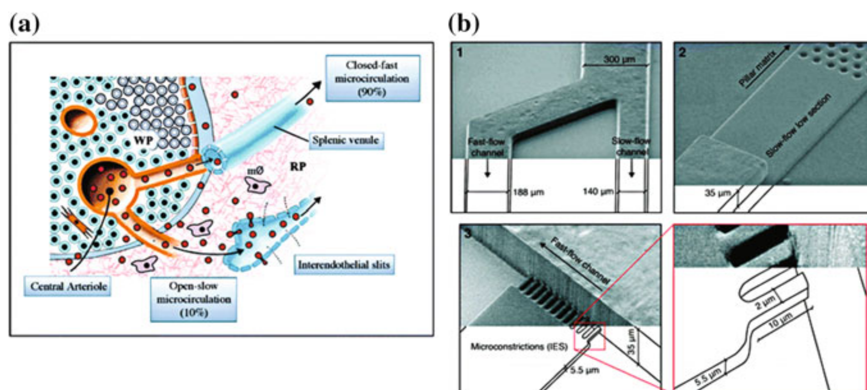

Fig. 4.6 *Spleen-on-a-chip* system: **a** diagram of the human spleen showing the closed-fast and open-slow microcirculations as well as the interendothelial slits (IES); **b** the fabricated microchannels within slow-flow channel to mimic IES. Reprinted with permission from Rigat-Brugarolas et al. (2014). Copyright 2014 Royal Society of Chemistry

consists of spleen pulp (white and red) and the marginal zone. The filtration effectiveness is due to slow blood microcirculation through the reticular meshwork of the spleen red pulp, which accompanies the increasing hematocrit. Next, specialized macrophages recognize and destroy pathologically changed red blood cells. To develop spleen in vivo-like model in the microsystem, all of the above spleen features should be taken into consideration. For example, Rigat-Brugarolas et al. (2014) have proposed a *Spleen-on-a-chip* device, which reconstructs hydrodynamic forces as well as physical and filtering properties of the minimal functional unit in spleen red pulp (Fig. 4.6).

Additionally, the proposed model gave the ability to maintain blood surveillance functions. To mimic specific microcirculation, two main microfluidic channels allowing physiological flow were designed. The pillar-based matrix, analogues to the reticular mesh, was created to mimic the hematocrit increase. The microsystem was used for investigation of new and old red blood cells, as well as cells infected by *Plasmodium vivax*. The obtained results confirmed that *Spleen-on-a-chip* system could be applied as the model for testing different types of red blood cells in both: physiologically proper and pathologically disturbed.

4.2.1.5 Brain-on-a-chip

The brain is one of the most complex and exquisite organ in the human body. Structural and functional hierarchy as well as high specialization are the specific features of the brain. Therefore, the identification of the minimal structural unit recapitulating the complexity and functions of the brain and central nervous system, even represented as a single neurotransmitter, might be potentially challenging

(Millet and Gillette 2012). Numerous processes and functions such as angiogenesis, immunological functions, or remodeling occur due to highly effective synaptic transmission through the communication of parenchyma cells within the extracellular matrix. The observed phenomena are tightly controlled by neural tissue in the central nervous system. Within the parenchyma of neural tissue, a variety of different cell types can be distinguished: primary synaptic effectors (neurons), astrocytes, microglia and oligodendrocytes (Abbott et al. 2006; Achyuta et al. 2013; Benarroch 2007).

Some brain models based on circular microfluidic coculture systems were described in the literature (Kilinc et al. 2011; Odawara et al. 2013). The structure of cerebral cortex consists of six layers with complex directional growth and multilayer connections mimic neurodevelopment. Because of that, the mimicking of specific brain microarchitecture with high accuracy requires the use of a 3D spatial model. The reconstruction of the cerebral cortex structure through control of the positions of the somata and the direction of neurite outgrowth on the basis of the orientation of collagen fibers was studied in the microsystem (Odawara et al. 2013). 3D spatial model of the brain can also be based on the cellular multilayer, obtained in alginate enriched agarose scaffolds. A novel microsystem was proposed for studying possible mechanisms of axon degeneration and death processes under control of space and specific molecular pathways (Kilinc et al. 2011). A scheme of a *Brain-on-a-chip* system is shown in Fig. 4.7. The microsystem was integrated with a porous PDMS membrane, which enabled the investigation of interaction between vascular (human brain microvascular endothelial) and neural (human neuronal and glial) cells.

Spatial arrangement of neural cells and 3D broad neural networks can be mimicked in the microsystems using neuro-spheres and spheroids networking

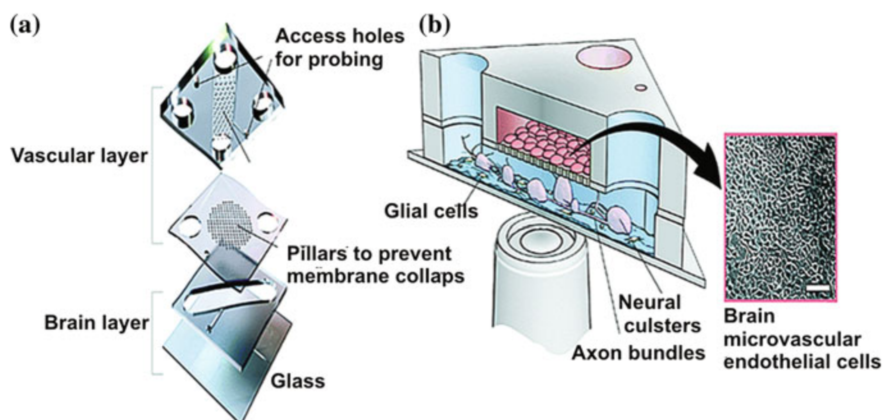

Fig. 4.7 **a** A scheme of *Brain-on-chip* system layers. **b** Cross-section of final *Brain-on-chip* platform showing culture of human neuronal and glial cells, interacting through a perforated membrane with a monolayer of human brain microvascular endothelial cells. Reprinted with permission from Kilic et al. (2016). Copyright 2016 Royal Society of Chemistry

(Jeong 2014; Kato-Negishi et al. 2013). Such models might be a tool for the analysis of morphological and functional changes of communicating neurons in order to study higher order neural networks between various brain regions. The microsystems with microgrooves can also be used for directing, isolating, and biochemical analysis of central nervous system axons.

3D organoids and multilayers also play a crucial role in the development of *Brain-on-a-chip* systems (Eiraku et al. 2008; Lancaster et al. 2013; Mariani et al. 2012). The reconstruction of various discrete regions of the hindbrain, midbrain, forebrain, as well as choroid plexus is realizable due to the formation of 3D organoids. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are used for generating three germ layers to form 3D polarized neuroepithelial structures. For example, Lancaster et al. (2013) developed bioreactor for neuroectodermal tissue generation and more complex tissue growth. The proposed system was based on a human pluripotent stem cell-derived (hPSCs-derived) 3D organoid culture, embedded in droplets of Matrigel. *Brain-on-a-chip* models, based on 3D spatial cultures and organoids from different genetic origin, have great capabilities in studies on human nervous system disease mechanisms and as a potential replacement for animals in preclinical trials.

4.2.1.6 Liver-on-a-chip

The liver is responsible for the detoxification processes, plasma protein synthesis, and glycogen storage. The liver is an organ with a highly complex structure. It contains hepatic lobules, formed of sinusoids and blood vessels lined with a permeable endothelium and surrounded by polarized hepatocytes and numerous mesenchymal cells (hepatic stellate cells (HSCs), macrophages, lymphocytes, and Küpffer cells). Hepatocytes are essential for ADMET (absorption, distribution, metabolism, excretion, and toxicity) model. These cells are closely related with liver functions. Therefore, this type of the cells is used for investigation of cytotoxicity mechanisms of novel pharmaceutical substances as well as the metabolism of xenobiotics. The examples of the microtechnologies used in studies on liver functionality and their applications are shown in Fig. 4.8.

One of the most commonly applied methods for hepatocyte activity amplification is their coculture with fibroblasts and nonparenchymal liver cells, i.e., endothelial, hepatic stellate, or Küpffer cells (Cho et al. 2010; Du et al. 2014; Kane et al. 2006; Kang et al. 2015; Khetani and Bhatia 2008). It allowed a specific intercellular communication to be investigated. For example, a miniaturized coculture platform based on 24-well plates was used for investigation fibroblasts surrounding the human hepatocytes. The hepatocytes formed well-organized colonies on micro-patterned islands coated with collagen. The importance of communication between coculture cells by canalicular transport, phase I/II metabolism, assessing gene expression profiles, secretion of products specific to liver function, as well as susceptibility to hepatotoxins was tested in the developed system (Khetani and Bhatia 2008). In comparison to models based on hepatocyte

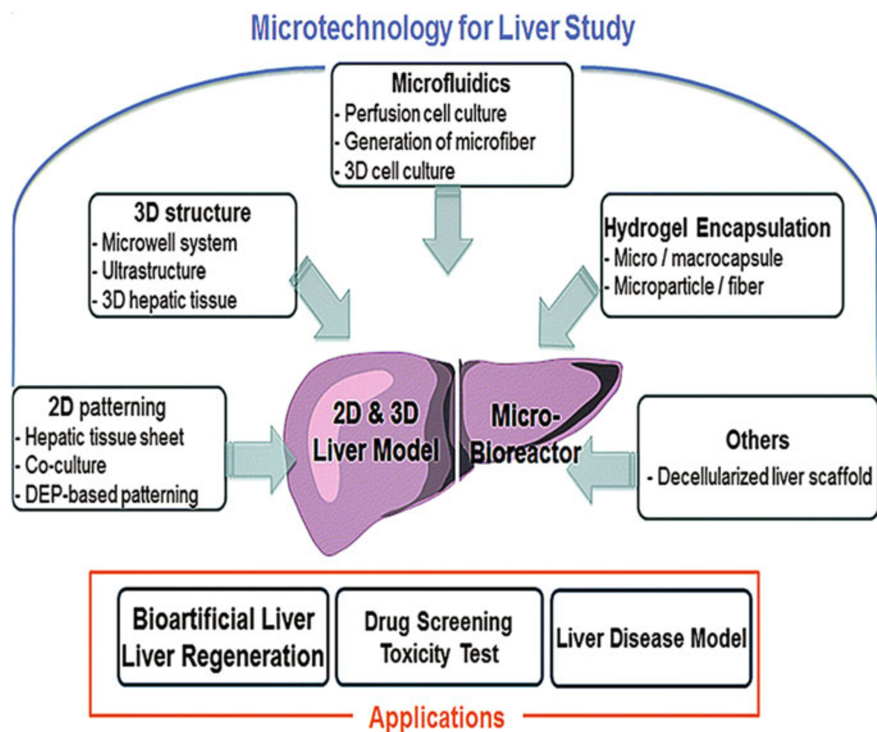

Fig. 4.8 Examples of the microtechnologies used in studies on liver functionality and their applications. Reprinted with permission from No et al. (2015). Copyright 2015 Royal Society of Chemistry

monoculture, the coculture models consisting of at least two cell types mimic hepatic function with higher efficiency. Du et al. (2014) reported encapsulation of hepatocytes with endothelial cells, differentiated from hiPSCs with the presence of recombinant protein. The cells were placed in fibers of multicomponent hydrogel and organized into 3D-patterned endothelialized liver tissue structures. The microsystem was composed of two PDMS chambers separated by a 10 μm-thick polyethylene (PE) membrane containing pores with 0.4 μm diameter (Fig. 4.9). The positive impact of stellate cell attendance on hepatocyte detoxification functions and the increased albumin secretion in a coculture with Küpffer cells were demonstrated. The relevant development in functionality of hepatocytes was determined in cocultures with cells derived from endothelium. Improved vascularization of the fiber scaffold by implanting it in a mouse model of partial hepatectomy was observed.

A hepatocyte, hepatic stellate cell, and endothelial coculture model enabled the assay of hepatic functions under static as well as perfusion conditions (Sumii et al. 2012). The flow loading also influenced hepatic function improvement (Ho et al. 2013; Prodanov et al. 2015). Cellular aggregates and spheroids were successfully

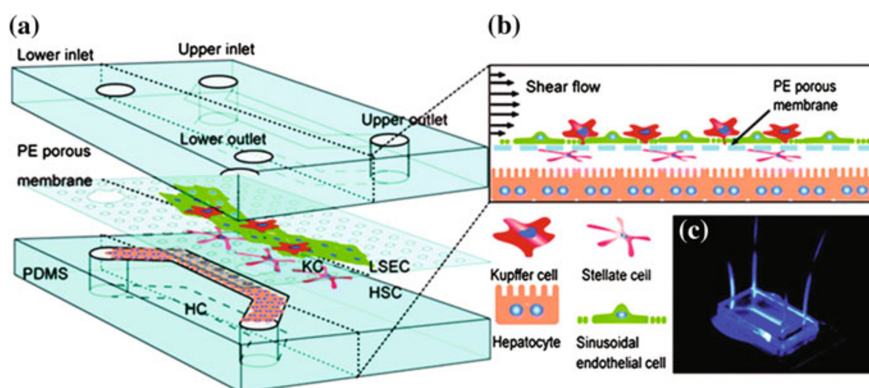

Fig. 4.9 **a** A scheme of the in vitro 3D liver sinusoid liver chip. **b** A scheme of 3D assembling. The four types of hepatic cells: liver sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, and hepatocytes were distributed layer-by-layer in a 3D manner. **c** A photographic image of an in vitro 3D liver sinusoid liver chip. Reprinted with permission from Du et al. (2017). Copyright 2017 Royal Society of Chemistry

used for better mimicking of liver functions (Chu et al. 2009; Feng et al. 2009; Lee et al. 2013; Schutte et al. 2009, 2011). Wong et al. (2011) developed a microwell platform for quantitative and qualitative analysis of the formation processes of liver cell-based spheroids. The intercellular communication was also examined in this chip. Monoculture of primary hepatocytes as well as coculture of primary hepatocytes and hepatic stellate cells was tested in the microsystem. The investigations of cell metabolism in such aggregates showed increased cytochrome P450 enzymatic activity and albumin secretion in the heterospheres in comparison to hepatospheres. Feng et al. (2010) developed a microdevice for hepatocyte spheroid culturing based on an electrospinning technique. The described device enabled aggregates to be obtained which exhibited cell fiber structures and functionality specific for the organ. A nanofiber scaffold-based bioreactor for inducing hepatocyte spheroids to assemble with galactosylated chitosan electrospun fibers was also proposed (Chu et al. 2009; Feng et al. 2009).

The next approach to the investigation liver in a microscale is mimicking of a liver injury-on-a-chip (Zhou et al. 2015). For this purpose, the authors developed the microsystem, which consisted of two chambers for coculture hepatocytes with stellate cells and three additional microchambers integrated with miniaturized electrodes for electrochemical monitoring of liver cell response. Alcohol-related hepatotoxic effects were tested in the microsystem. The simulation of a liver sinusoid with a microfluidic artificial endothelium barrier was also developed in the microsystems. For this purpose, packing a high density of hepatocyte suspension into a microchannel is used to create liver acinus-like mass transport properties (Lee et al. 2007). Densely packed hepatocytes were fed by diffusion of nutrients across the parallel carrying microchannels. The described solution mimics the numerous mass exchange characteristics of functional liver sinusoids such as: defined tissue

and fluid transport regions, continuous nutrient exchange, and extensive intercellular contact. The microsystems are also utilized for hepatotoxicity assays (i.e., diclofenac). The novel categories of liver sinusoid models based on perfusion channels and separated chambers for cell culturing were developed with the presence endothelial-like physical barriers. This class of liver models was called an integrated microphysiological analysis platform (iMAP) (Hong and Lee 2014). The iMAP for an iPSC-derived hepatocyte model offers a lot of benefits like the possibility of obtaining enhanced drug metabolism and protein synthesis with the avoidance of polarized hepatocyte dissociation, assembling into spatial sinusoidal structures as well as facilitated organogenesis-like tissue development.

Further directions for research on modeling of fully functional artificial liver microarchitecture might be developing the space between blood sinusoids on a background of fenestrated endothelium and hepatocytes as well as basolateral villi. Domansky et al. (2010) proposed a microplatform enabled hepatocytes with stellate, Küpffer, and liver sinusoidal endothelial cells to be cultured under continuous perfusion by diaphragm micropumps integrated with a multiwell plate format. The system consisted of bioreactors separated by fluidic channels and containing scaffolds at densities comparable to normal tissue densities and specifies the functional zonation of hepatocytes. A prediction of optimal parameters for primary liver cell culturing might be established by continuously adjusting oxygen consumption and transport in the circulating culture medium. The described microplatform also allows the regulation of flow rates based on oxygen consumption and long-term steady maintenance of the oxygen gradient. Because of the highly accurate mimicking of complexity of liver sinusoid functionality and responses to numerous stimuli, this in vitro model is a promising tool for studying the liver metabolism and biological activity of novel pharmaceutical substances.

4.2.2 Cells Sources

One of the most meaningful issues during the process of designing *Organ-on-a-chip* models is the proper choice of cell sources. Animal tissues, widely used as the primary cell sources in most current experimental models, are distinguished by significantly different physiology and pathophysiology in comparison to a human's. What is more, models based on cell cultures and tissues of animal origin do not represent most human diseases and pathological conditions (e.g., neurological and autoimmunological disorders) adequately. In consequence, the investigations utilizing human cell sources with numerous inherent genetic variations are requisite (Boughton et al. 2011; van der Meer and van den Berg 2012).

There is a wide range of possible cell sources as tissues cultured under ex vivo conditions, primary cultures, stem cells, and immortalized, commercially available cells. One of the main requirements of cell sources is their ability for high reproducibility and reliability on pharmaceutical applications such as screening research and simultaneous research on a lot of novel, potentially therapeutically effective

substances (Huh et al. 2011). It is worth noting that each possible cell source has limitations. Immortalized cell lines, the most commonly established for studies on the in vitro level, are distinguished by homogenous and non-patient-specific genomes as well as by phenotype and mismatches with whole tissue. For comparison, acquisition and further culturing of the patient-specific derived from patient tissues and genetically nonhomogenous primary cells are potentially problematic (van der Meer and van den Berg 2012). The availability of ex vivo cell sources, except for tumors, is limited. Moreover, the assays based on this type of cell source must be performed within several hours due to the fact that there are rapid changes in cell function (Verpoorte et al. 2015).

Recently performed studies have brought great development in functional stem cell production technology with the use of genetic manipulation (Giobbe et al. 2015). However, the elaboration of proper protocols for stem cell differentiation under controlled conditions, with avoidance of chromosomic aberrations or epigenetic DNA changes, might still be a challenge. Stem cell technology offers a lot of potential benefits for research possibilities on neurological genetically inherited and cardiac diseases and creating novel platforms for personalized medicine (Wagner and Ho 2007).

4.3 *Human-on-a-chip*—The Integrated Multi-organ Platforms

The human physiology and the organism's functionality under pathological conditions as well as the response to the tested treatment are the results of interaction between different organs. Microfluidics is one of the most common strategies for physiological studies on the integration of cell culturing sections, each representing major organ-level functions (Blake et al. 2010; van Midwoud et al. 2011a, b). This approach allows the metabolism of therapeutically effective substances and the absorptive events as well as the response to electrical stimulation to be monitored. On the other hand, the limitation of these specimens' viability as well as the lack of normal chemical and mechanical stimulus in the described studies precludes the use of this approach in further research on possible toxic and side effects of the drugs (van Midwoud et al. 2011a). As an alternative solution, Esch et al. (2011) have proposed a *Body-on-a-chip* model. The described device was based on a system of multiple microchambers (each one contains a different type of the cells) connected by a network of microfluidic channels that permit recirculation and exchange of metabolites in a physiologically relevant manner (Fig. 4.10). The main limitations of the first variant of such approaches were the lack of optimal tissue-specific differentiation or organ-relevant microenvironments. By replacing the 2D analog cell culturing models with a more complex, spatial model based on hydrogel, it was possible to obtain more physiological accurate conditions. The human cells suspended in hydrogels (e.g., Matrigel, alginate solution) were gelled in the

microdevice. In consequence, three interconnected compartments with 3D tissue constructs (liver, bone marrow, a colon tumor) were formed. Such a solution, in contrast to the conventional cell cultures, enabled efficacy, hepatotoxicity, and hematological toxicity to be simultaneously observed as the effects of exposition to an anticancer drug (Tegafur). The results obtained were comparable to the effects observed under in vivo conditions. In the next steps, the described approach was developed by replacing the external pumps with the operation using gravity-induced flow as well as by the use of a more advanced mathematical model of the pharmacokinetic and pharmacodynamic (PK/PD) profiles of the anticancer drugs to predict the experimental data (Imura et al. 2010; Sung et al. 2010). Analogous solutions were also used as a tool for studying the biological activity of anti-breast cancer drugs in the presence of liver and intestinal models which represented human metabolism and hepatotoxicity (Imura et al. 2010).

4.4 Future Directions for Research

There are numerous challenges to overcome before *Organ-on-a-chip* systems will be widely used for biochemical applications. The greatest limitation of such systems is the problem of precisely controlling of specific microenviroment for human cell differentiation and maturation. The lack of compatibility between microdevices (owing to their small dimensions and small sample sizes) and conventional biochemical and measurement techniques hinders downstream and online analysis of biological parameters. What is more, in current laboratory practice, most of the cell

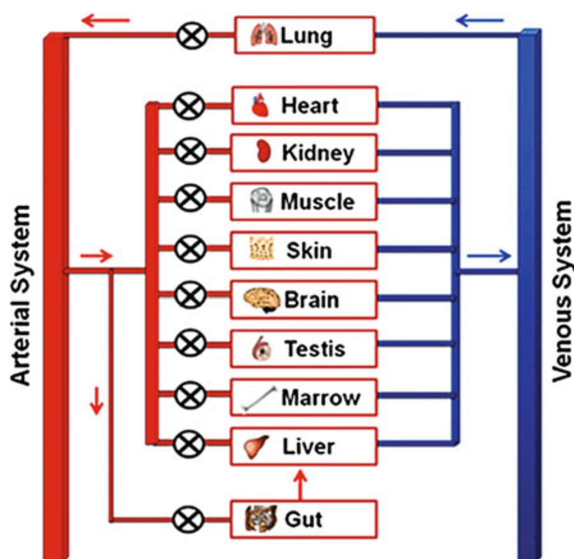

Fig. 4.10 A scheme of *Human-on-a-chip* system. Reprinted with permission from Huh et al. (2012). Copyright 2012 Royal Society of Chemistry

culture substrates are based on synthetic materials which cannot imitate the ECM adequately. One of the most common strategies is the use of chemical surface modification to minimize the effectiveness of absorption of small organic compounds (e.g., drug molecules) in widely used PDMS. Despite their limitations, *Organ-on-a-chip* systems have great potential for becoming a tool for drug or alternative therapies discovery.

Acknowledgements This work was financially supported within a frame of OPUS 11 program No. UMO-2016/21/B/ST5/01774 and Warsaw University of Technology.

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