Towards Human on a Chip: Recent Progress and Future Perspective

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Abstract: A system of multiple organs integrated on a single chip or human on a chip (HUC) has a great potential for drug discovery. Such a system helps to advance the fundamental understanding of diseases as well as the complex interactions between cells, tissues and organs. HUC models will potentially overcome the shortcomings of traditional animal models such as high cost, incompatibility with human physiology, the inability to control and manipulate the microenvironment in vivo and the lack of efficacy. The matching length scale of biological structures and micromachined components makes a microfluidic chip the ideal platform to investigate physiological events. This paper presents an overview of the state of the art of the development of HUC. The paper also provides a perspective on the integration of cell culture on a chip to create an ethical human model and to provide insights into the sensitivity of different cell constructs on drugs.

Keywords: Cells, human on a chip, lab on a chip, microfluidics, organ on a chip.

1. THE QUEST FOR A RELIABLE HUMAN MODEL

The discovery of new drugs for the treatment of human diseases requires a reliable model. Due to ethical concerns, initial investigations on the effect of a new drug and its interactions at the molecular and cellular level are often carried out in animal models. Besides ethical problems, the use of animal models also faces financial hurdles, as running an animal facility is relatively expensive. The cost involved is not only for the maintenance of the animals, but also for the security and safety of the facility. The major drawback of an animal model is its incompatibility with human physiology. Animal models do not always detect the toxic effects of drugs manifested in humans. Furthermore, drugs that are toxic to animal may not be toxic to humans, leading to the loss of possible promising drug candidates [1]. Vice versa, drug candidates tested to be effective to animals may not work in humans. For example, when studying the endocrine system, a glucose-dependent insulinotropic polypeptide stimulates glucagon-like peptide-1 (GLP-1) in rodent models, but are ineffective on human cells in vivo [2-4]. Furthermore, systematic studies through observation of biological changes are limited by the inability to control and manipulate the microenvironment in vivo [5]. Due to the lack of efficacy in animal models or toxicity which was not revealed during pre-screening, only one in nine drugs entering clinical trials will finally get approved [6, 7].

Recently, a number of excellent review papers on mimicking human organs have been published. Multi-organ microdevices can model human metabolism, including the therapeutic and toxic effects of drugs. Esh et al. [8] reviewed the recent development of multi-organ microdevices and their capabilities in studying of drug action. Design considerations for mimicking human metabolism were also discussed. Chan et al. [9] reviewed the recent development of organs-on-a-chip devices from an engineering perspective including microfabrication and cell patterning technologies. Bhatia and Ingber [10] recently gave a perspective on the development and applications of organs-on-a-chip devices. The review focused on the design and fabrication of microfluidic cell culture devices that simulate tissue- and organ-level physiology.

2. MIMICKING THE IN VIVO MICROENVIRONMENT OF CELLS

Every cell type needs its own microenvironment. The microenvironment is a specific set of chemical and mechanical conditions, which influence and regulate the cell fate. In these conditions, a cell constantly senses the various inputs such as soluble factors, cell-matrix interactions and cell-cell contacts, as well as other parameters such as pH, oxygen concentration and shear stress [11]. Upon sensing these inputs, cells process the information and adapt themselves to the current surrounding. Therefore, understanding the key inputs of the cellular environment is important for creating physiologically acceptable cellular conditions in a microfluidic device.

2.1. 3D Cell Culture

Traditionally, cells are grown in Petri dishes and form a two-dimensional (2D) culture. The cells spread out on the surface of the dish, often in a single layer. This 2D morphology does not reflect the three-dimensional (3D) in-vivo state of the cells, having a different expression pattern and altering
the cell’s function and response, Fig. (1) [12]. Microfluidic technology allows the implementation of a 3D culture, which mimics the in-vivo state of cells [13]. Cells in a 3D microfluidic culture chip (3D-μFC) retain their 3D morphology and show a higher functionality than the 2D counterparts [14] as well as a higher sensitivity towards drugs, Fig. (2) [15]. The precise control of the microenvironment plays an important role in mimicking the in-vivo conditions. Microfluidic technology allows for a higher level of control of microenvironments as compared to petri dish cultures. The technology enables the control over chemical and mechanical cues such as media composition, growth factors, surface rigidity, surface texture, sheer stress, and secreted cues from other cells.

Fig. (1). Ex-vivo tumor models. Microscopic images of monolayers and spheroids of human mesothelioma cell line NCI-H226. 2D: monolayers; 3D: spheroids. Scale bar: 400 μm. Microarray analysis revealed that 142 probe sets were differentially expressed between tumor spheroids and monolayers (from [12], with permission from PLoS One).

In addition, microfluidic technology and the development of μFC make pharmacokinetics more predictive [16]. These microfluidic devices can potentially mimic the in-vivo physiology more accurately, providing physiologically more realistic parameters. For example, in-vitro static hepatocyte cultures, which are used as a screen in pre-clinical studies, have serious limitations in predicting the human liver response due the loss of liver specific functions [17, 18]. However, 3D hepatic cell cultures in a microfluidic device preserve some of the liver functions because of a more realistic microenvironment [19-21]. The improvement can clearly be observed by the higher level of sensitivity towards drugs as compared with a static culture, Fig. (2c) [19]. A step closer to a more realistic model is the integration of multiple cell types to form co-cultures, as many physiological responses depend on their interaction with neighbouring cells. In the case of hepatocytes, the interaction with the sinusoidal endothelium improves the predictability by inducing vascularization of the 3D culture [22, 23].

The physiological model of organs can be further improved by including pharmacokinetic parameters such as the organ size ratio, residence time and the relative flow rate. Each organ is represented by their specific cell type and compartmentalised on a single microfluidic chip. The organ size is modelled by the compartment size, while the fluidic resistance of each compartment determines the flow rate and flow distribution. Depending on the cell types, this system provides a more accurate physiological environment than the conventional static culture plates [24]. Combining the in-vitro with in-silico approaches will open a new direction to physiologically based pharmacokinetic (PBPK) modelling, with improved predictability of drug candidates [25].

2.2. Chemical Environment

The chemical environment of cells includes soluble factors such as growth factors and cytokines as well as extracellular matrix (ECM). Growth factors stimulate cellular growth, proliferation and differentiation [26]. Cytokines regulate wound healing [27], chemotaxis [28], and angiogenesis [29]. The microfluidic environments enable cells to retain endogenous growth factors, thereby favouring their proliferation [30, 31]. Therefore, concentration and gradient [32] of soluble factors in the in vivo tissue microenvironment of an organ should be carefully controlled in a human on a chip. An effective method to deliver soluble factors locally to the cells is using gelatine microspheres [33].

Similar to soluble factors, ECM has profound effects on regulating cell behaviour and orchestrating cell functions during the formation of tissue and homeostasis [34]. Integrin as a transmembrane receptor mediates the attachment of a cell to the ECM. Through this attachment, integrins transmit information about the chemical composition such as ECM specificity as well as its mechanical status such as density, elasticity, geometry and surface topography, which alter the shape of the cell and its cytoskeletal organization [35]. Due to its importance, ECM has been a major target of manipulation in both conventional 2D and the-state-of-art 3D cell culture. Among the various methods for ECM manipulation, micro contact printing (μCP) is the most widely used technique for reconstructing the cellular microenvironment [36]. μCP is a soft lithography technique that uses a moulded polydimethylsiloxane (PDMS) stamp to transfer ECM to a substrate [37]. The stamp is first coated with ECM protein and then transfers it to a substrate through contact. Foehl and Toner [38] provided an extensive review on the different patterning methods of ECM. Similar to soluble factors, ECM should be carefully engineered to reconstitute the in-vivo tissue microenvironment.

2.3. Mechanical Environment

Another consideration is the mechanical environment for engineering the in vivo tissue environment in a small scale. In contrast to cells grown on a conventional culture dish, most cells in our body live in a dynamic microfluidic condition, where cells take oxygen and nutrients from the laminar flow of blood. How can we mimic the in vivo mechanical environment of cells? The following discussion on the key differences between a culture dish and the in vivo tissues will provide the answer for this question.

2.3.1. Fluid-to-Cell Volume Ratio

In a conventional culture dish, the volume of fluid is much greater than that of the cells. Thus, soluble factors released from cells dissipate quickly in the medium. Since the majority of the volume within an in vivo tissue is the cell volume, soluble factors from cells can influence neighbouring cells and themselves with negligible dissipation. As a result, endocrine and paracrine effects in in-vivo tissues are
Towards Human on a Chip: Recent Progress Micro and Nanosystems, 2014, Vol. 6, No. 4 217

Fig. (2). The concept of a 3D cell culture chip. (a) An array of 200-µm pillars with 20-µm gaps retain the cells. The medium is perfused from both sides of the pillar array. (b) Microscopic image of the 3D culture of hepatocytes after 72 hours. (c) Drug sensitivity of a 3D perfusion culture as compared to that of a 2D static culture. The graph shows the viability of primary rat hepatocytes as function of the drug concentration [15].

more significant than those in a culture dish. In this regard, microfluidic cell culture devices are highly suitable for inducing endocrine and paracrine effects, which play an important role in cell proliferation, signalling, and chemotaxis [39]. The low fluid-to-cell volume ratio in a tissue suppresses the dissipation of soluble factors by uncontrolled convection.

2.3.2. Flow

The in-vivo tissue environment has a continuous-flow condition. Many types of cells prefer the continuous-flow condition to the static one. The continuous flow transports oxygen and nutrients to the cells and washes away soluble factors. For instance, differentiated cells such as hepatocytes and lung cells require a continuous-flow condition for their growth [40]. However, stem cells such as adipose tissue-derived stem cells (ATSCs) prefer the static condition to reconstitute their hypoxic niche in vivo [41].

2.3.3. Mechanical Stimuli

Mechanical stimulus is another important factor to be considered in designing human-on-a-chip devices. In in-vivo tissues, cells usually experience dynamic mechanical stimuli such as fluid shear stress, tension and compression. Each organ has its preferential mechanical stimuli. For instance, renal tubular cells in the kidney are exposed to shear stresses exerted by the flow of blood and urine. The cells need the shear stress to differentiate and function [42]. Recently, Huh et al. [43] demonstrated that lung epithelial and endothelial cells grown on a chip cyclic require mechanical stretching to immunologically respond to nanoparticles and bacteria. Similarly, Kim et al. [44] showed that mechanical strain enhances the function of human Caco-2 intestinal epithelial cells to form villi-like structure with a physiologically relevant intestinal barrier functions. However, stem cells and hepatocytes do not like the shear stress [41]. Therefore, if different organs are integrated into a single chip, mechanical stimuli should be addressed individually to meet their needs.

3. BACKGROUND OF ORGAN ON A CHIP

Culturing and connecting more than two cell types together on a single device lead to a more sophisticated model that can partially mimic human physiology. Compared to an in-vivo system, an organ-on-a-chip (OOC) has several ad-
vantages. For instance, such as system proved to be useful for determining the half maximal inhibitory concentration (IC50) value of drugs [15]. The IC50 represents the capability of a drug to inhibit a specific biological or biochemical function. Furthermore, an OOC system provides information, which is not accessible in in-vivo systems due to difficulties in instrumentation. An OOC system is useful for evaluating drug mixtures targeting single-drug resistance diseases [45]. An OOC device can model a specific disease as well as a personalised disease. For instance, cells could be taken from a person’s skin and reprogrammed into a cell type specific to this person [46]. The toxicity and efficacy of a drug can then be tested specifically for that person.

3.1. Organs in Bottles

Cell cultures mimicking human physiology did not start with microfluidics [47]. The first compartmentalised coculture for toxicity screening can be traced back to the work of Riley et al. [48]. This system consists of 2 Teflon compartments separated by a semi-permeable membrane. One compartment holds the drug metabolising system, while the other contains the target cells. To prove the device concept, the drug Dapsome was incubated with human microsomal protein and NADPH in the chamber with metabolising liver cells. Dapsome has no toxic effect on the liver cells, but causes 8.7% of the target cells (leucocytes) to die. This engineered system proved that although the drug was not toxic to the metabolising cells, it does affect target cells due to a toxic metabolic product of the drug. This work shows the significance of in vitro toxicity screening, as animal testing does not clearly reveal the side effects. Furthermore, the human cells also lead to a more accurate predictive model for drug screening.

The next step of the above concept is combining multi-compartment computer models with single compartment in-vitro cultures to simulate more human physiological conditions [49]. For instance, the toxic effect of naphthalene metabolites to lung was shown by using milk dilution bottles (a square-sided bottle of 160-ml volume that is commonly used for dilutions) for the liver and lung cells, and a glass reaction beaker to represent other cells. A set of pumps controls the residence time in the three compartments (Fig. 3a). Connecting the water-jacket of the reaction beaker to a water bath keeps the whole system at 37°C.

Viravaidya et al. [50] integrated the bioreactors into a silicon wafer using micromachining technology to miniaturise the above multi-compartment system, Fig. (3b). While the cell to liquid volume ratio in the bottle was less than 1:1000, far exceeding the physiological levels, the on-chip volume ratio was 1:1 [50]. The residence time and flow rates were determined by the size of the compartments and the flow resistance in the connecting channels. In this setup, only the liver and lung compartments had cells. The other compartments were used for flow distribution [50]. This system was able to show that the metabolic products of naphthalene caused glutathione depletion in the lung cells. The same result was also shown in the experiment reported by [49], indicating that microfluidics can be applied to drug toxicity assays, but using much less cells and reagents.

3.2. Organs in 3D Cell Cultures

The next advancement toward developing an OOC system was the integration of 3D culture in hydrogel [24]. Compared to cells grown in a 2D culture, those grown in a 3D culture show improved functionality and higher sensitivity towards drugs. The higher level of complexity in 3D cell cultures contributes to a more in-vivo-like response. However, a simple 3D culture is far from mimicking a real organ. A 3D cell culture requires interactions between different cell types and a better control of their microenvironment to model a real organ. For instance, hepatocytes in the liver tissue make up a single cell layer around the bile ductile which in turn flanks the sinusoid separated by a single layer of endothelial cells. In contrast, a 3D culture is basically just a clump of cells. Drug toxicity test showed different results from a clump and a single layer of cells in a 3D-like structure. The architecture of the cell culture is here the key factor, as cells do respond to their physical and spatial surround-
ing. Thus, liver on a chip as a typical OOC has progressed from a simple 3D-cell culture in a microchannel to something that resembles the shape of a basic liver structure. This shape can be formed by either dielectrophoresis [51] or micromachining [52] mimicking the permeable endothelium layer between the hepatocytes and the sinusoid.

Culturing different cell types in a single compartment improves their interaction. A micro-patterned co-culture with hepatocytes and fibroblasts maintained the functionality of the hepatocytes for more than 4 weeks [21]. In contrast, the continuously diminishing functionality of pure hepatocyte cultures lasted only for 2 weeks. A collagen/marigel sandwich culture could also maintain the functionality of hepatocytes only for 2 weeks [53]. These works show that co-cultures have a positive effect on the cell functions. The pancreas is another example of a system with more cell types, of which the α- and β-cells are the most important [54]. Together as an ensemble, the two cell types regulate part of the glucose levels in the blood. Therefore, they should be cultured together to form a model for investigating insulin production and glucose regulation.

Besides the 3D environment and co-culture of different cell types, the physical location of cells also plays an important role in mimicking the real organ, as some cell signals have spatiotemporal limits. For instance, the distance between the two different cell types within a co-culture determines the fate of the soluble signal. Culturing hepatocytes and stromal cells in a reconfigurable compartment elegantly tested this hypothesis [55]. Mechanically increasing the distance between the hepatocytes and stromal cells decreased the functionality of the hepatocytes.

### 3.3. Organs on a Chip

In anatomical terms, an organ is a collection of tissues joined together to perform a given function [56]. The combination of 3D-cell cultures with microtechnology and the integration of chemical and mechanical cues led to the development of more complex. This section reviews the state of the art of OOC devices. These devices may not necessarily mimic an entire organ, but at least model some specific functions of an organ. The majority of these devices were made of polydimethylsiloxane (PDMS) using soft lithography technology introduced by Whitesides’ group [57]. PDMS is cast against a micromachined mould. After peeling off from the mould, fluidic ports are opened. The PDMS part is then bonded to a glass substrate, usually a microscope slide. Only a few devices are fabricated in glass, because the micromachining process of glass is a more laborious and expensive. At the end of the following subsections, we list the cells used in the perspective OOC devices.

#### 3.3.1. Brain

The brain is the most vascularised structure in the body. Each neuron is only one cell away from a blood vessel [58]. Of great interest is a model of the blood-brain barrier (BBB), which is an intricate network of vascular endothelial cells isolating the central nervous system from systemic blood circulation (Fig. 4). The main cells in a BBB are astrocytes. Astrocytes provide biochemical support to endothelium cells as well as nutrition to neural tissue, endothelial cells, and pericytes, which among others regulate the capillary blood flow and permeability of the BBB. A model of the BBB allows for the detailed study of the drug passage and of any impairment caused by the drug. In the following examples, only the endothelial cells were used. The tightness of the junctions between the endothelial cells is a defense mechanism to prevent the transport of toxic substances, which would otherwise damage the brain tissue. Unfortunately, therapeutic drugs are also prevented from entering the brain.

A BBB model was formed by creating a channel with cerebral endothelial cells separated by a barrier with micro gaps connecting a central chamber filled with astrocytes (Fig. 5) [59, 60]. Instead of constructing microgaps as shown in Fig. (5), endothelium cells can also be cultured on a 10 μm thick polycarbonate membrane with a 0.4 μm pore size. In a BBB device, a suitable method is needed to monitor drugs that specifically target the brain and must pass through the junctions between the endothelial cells. The tightness of the junctions can be quantified by measuring the transendothelial electrical resistance (TEER) [61]. Measuring a change in the electrical resistance reveals if a drug affects the junctions. Griep et al. [61] showed that the addition of a small cell signaling protein, the inflammation cytokine tumour necrosis factor α (TNF-α) reduces the TEER value by 10 times. In contrast, the shear stress increase the TEER value by 3 times, meaning that shear stress tightens the junctions.

Since fabricating a structure as complex as the brain is a great challenge, it makes sense to culture an entire slice of brain on a chip [62]. As the slicing process kills a layer of cells, sharp micro-electrodes have to penetrate the dead layer to get closer to the living neurons [63]. The disadvantage of a slice culture is its short life span. Sliced tissue cultures can be maintained for up to 5 days in the above study, while cell based cultures usually last beyond 14 days. The cells cultured in the above devices usually belong to the cancer cell lines. However, induced pluripotent stem cells (iPSCs) have been recently introduced for brain on a chip applications [64].

**Cell used:** cerebral endothelium derived from iPSC; RBE4 (Rat Brain endothelial); HUVEC (human umbilical vein endothelial cells); hCMEc/D3 (Human Brain Endothelial Cell Line).

#### 3.3.2. Breast

A model of breast cancer is of great interest for screening a suitable anti-cancer drug. Despite the significance of breast
cancer, not many microfluidics-based models have been developed. A number of research groups investigated the migration of mammalian tumours in a 3D matrix [65]. These migration studies could give insight on the origin of metastasis. Chen et al. [66] screened anti-cancer drug on mammary epithelial cells 3D cultured in a perfused 96-well plate (Fig. 6). The culture was maintained for 9 days. Treating the cells with the anti-cancer drug Paclitaxel showed that the 3D culture had a higher cytotoxicity than a 2D culture. With the same drug concentration, more cells die in a 3D culture. The ductal system inside the breast tissue has been modelled by microchannels [67]. In this work, fluorescently labelled super-paramagnetic beads were pumped through the ducts to simulate mammary ductoscopy. This method could potentially be used to tag cancer cells preceding a surgery.

**Cells used**: MCF7 (human breast adenocarcinoma cell line); HMT-3522 S1 cells (human breast epithelial cell line series); MCF-10A (human mammary epithelial cells).

### 3.3.3. Eye

A few studies on retina cultures in microfluidic format have been reported. A recent study focused on the visual impairment caused by photoreceptor degeneration. The retina disease was modelled by a microchannel PLGA (poly(lactic-co-glycolic acid) scaffold with micro-columnar space to model the basic level of retinal organisation, mimicking what is observed *in vivo*, Fig. (8) [68]. Viability of the photoreceptors could be increased 18-fold by culturing the cells at the gas-liquid interface, as opposed to a fully submerged culture. The radial-columnar unit has a diameter in the range of 15-20 μm, which is necessary to facilitate the alignment of the retinal neurons. In this system, mouse and human embryonic stem cells were grown and exhibited several characteristics of retinal cells.

**Cells used**: retinal progenitor cells derived from hESC (human embryonic stem cell); retinae of E 6–7 chick embryos chick retinal ganglion cell (RGC).
Towards Human on a Chip: Recent Progress

Micro and Nanosystems, 2014, Vol. 6, No. 4

221

Fig. (8). Retinal model: (a) Dissociated neonatal mouse retinal neurons (Nrl-GFP) are layered on retinal pigment epithelium (RPE) explants; (b) A cross section of the Nrl-GFP rods; (c) A TEM image of the scaffold on the RPE layer; (d) A higher magnification of the scaffold cross section in a microchannel (from [68], reproduced with permission from Biomaterials).

Fig. (9). Heart model: (a) A crosssection of a micro-spherical heart-like pump. A sheet of cardiomyocytes is wrapped on to the fibronectin coated PDMS hollow sphere; (b) The engineered micro-heart (from [73], reproduced by permission of The Royal Society of Chemistry).

3.3.4. Heart

The heart is a powerful natural pump that delivers blood to the other organs. Thus, building a microfluidic pump from cardiomyocytes is an obvious step to mimic heart function. A pump powered by cardiomyocytes can be controlled by changing the culture temperature [69]. This heart model is self-actuating and requires only a chemical energy source (Fig. 9) [70, 71]. Integrating a heart as a pump in a microfluidic device provides an interesting tool for screening drugs against heart diseases. The effect of the drug can be directly monitored by the flow rate generated by the pump.

Recently, ventricular myocytes were cultured on top of an elastomeric thin film to measure the contractility, structure function and pharmacology [72]. A microfluidic platform as a disease model can characterise in real time the reactive oxygen species, which is a major factor in cardiac diseases [73] and cardiac contraction [74]. Fabricating a hydrogel support with nano-features allows for a highly organised function of the cardiac tissues constructs in the same manner as observed *in vivo* [75]. Changing these nanostructures results in change of the cell structure and function, opening up a new way to engineer an organ model on a chip.

Shimeck *et al.* [76] reported a multi-organ device that can mimic the transport function of the human cardiovascular system. The pulsatile fluid flow was delivered by an integrated peristaltic micropump. The system was able to create physiologically relevant shear stress on human dermal microvascular endothelial cells.

**Cells used:** neonatal rat cardiomyocytes (primary cells); neonatal Sprague- Dawley rat heart cells (primary cells); NRVM (neonatal rat ventricular myocytes)

3.3.5. Intestine

The most characteristic feature of the intestines is the villi structure that lines the apical side of the gastrointestinal tract, Fig. (10) [77]. Each villus has many microvilli that increase the absorption area of the intestinal walls by 30 to 60 fold (Fig. 10). The villi are around 1 mm high and 0.1
mm in diameter. Mimicking these villi and at the same time accommodating intestine epithelium cells in a hydrogel are real engineering challenges. Sung et al. [78] combined laser ablation and sacrificial PDMS moulding to form the hydrogel scaffold mimicking the villi, Fig. (11). Cells could either be encapsulated in the hydrogel or seeded onto the structure. In the latter case, the cells took 3 weeks to cover the hydrogel pillars.

Another method to model intestine is constructing micro-porous membranes in the shape of 3D-villi [79]. These artificial villi make culturing colon carcinoma cell line (Caco-2 cells) over a period of 3 weeks possible. Since the intestines are important for drug adsorption, drug molecules should be able to pass the tight junctions between the intestine endothelium cells. Caco-2 cells were cultured on top of a porous membrane inside a microfluidic chamber. The permeation of drug molecules was detected using integrated optical fibres [80]. Compared to a conventional in vitro cell culture system for studying the intestinal absorption of drugs, such a microfluidic system requires 80% less cells [81].

In vivo intestinal epithelium cells are covered with beneficial microbes, which contribute significantly to the barrier function of the intestines. Culturing microbes such as lactic acid bacteria on top of the Caco-2 cells represents a more realistic intestine absorption model [44]. Cyclic mechanical strain that mimics the peristaltic motion of bowels movements influences the cell functionality as well as the absorption rates. Culturing the cells on a stretchable porous membrane can simulate the induced strain [82]. Such a device can be used to capture the essential functions of the intestines and contribute to drug and toxicity screening.

Cells used: Caco-2 (colon carcinoma cell line).

3.3.6. Kidney and Spleen

The main function of a kidney is maintaining the homeostasis of the body by means of osmoregulation [83]. With the aid of the endocrine system, the permeability of the proximal convoluted tubule in the kidneys can be increased to reabsorb water and to prevent it from being excreted. As such, the kidney regulates the amount of waste in the urine. The shear stress caused by the fluid flow in the renal tubules plays a role in the regulation of the ions and water balance. Shear stress also contributes to the reorganisation and reformulation in the proximal tubular epithelial cells [84, 85]. Incorporating shear stress and controlling the topology in a microfluidic chip result in a tube-like environment which enhances the formation of tissue with native in vivo kidney physiology [86]. Compared to a flat surface, cells on topographical surfaces should increase nuclear alignment. Adding the shear stress using fluid flow increases the alignment of the topographical surfaces. Typically, renal cells are cultured on a porous membrane with fluidic compartments on both sides, Fig. (13) [87]. The cells are then exposed to the shear stress. The difference between static and perfused culture can be observed by imaging the tight junctions between the cells and the actin rearrangements [88, 42]. The effect of shear stress and the subsequent cell arrangements on trans- and paracellular transport can be studied after hormonal stimulations [42]. In this manner, albumin handling by renal proximal tubular cells was evaluated. Albumin processed by the cells was broken down in smaller fragments and excreted by the cells [89]. Compared to 2D cultures, the cells exhibited greater albumin transport and glucose reabsorption [90].

The spleen is a blood filter that has a similar structure to a lymphoid organ. The spleen removes senescent, damaged, or infected red blood cells. The two major areas of a spleen are the red pump and the white pulp. While the red pulp consists of filtration beds, the white pulp provides active immune response. Rigat-Brugarolas [91] mimicked the filtering function of the spleen on a chip. Using the device, mechanical and physiological responses on healthy human red blood cells and malaria-infected cells were studied.

Cell used: HK-2 (human renal proximal tubule cell line); IMCD (primary rat inner medullary collecting duct); OK

Fig. (11). Intestine model: (a) A scanning electron microscopy (SEM) image of a villi made in PDMS; (b) Confocal image of a collagen villi shaped scaffold; (c) Caco-2 cells on the collagen villi; (d) A SEM image of human jejuna villi; (e) A confocal microscope image of a slice of Caco-2 cells on the collagen villi (from [78], reproduced with permission from the Royal Society of Chemistry).
(Opossum kidney epithelial cells); RPTEC (human renal proximal tubule epithelial cells); MPT (Mouse proximal tubule cells); LLC-PK1 (Pig Kidney Epithelial Cells); Mouse PTC (proximal tubule cells).

Fig. (12). The kidney filters the blood in the nephron. There are 1-1.3 million nephrons in a kidney.

Fig. (13). Kidney model: (a) The cross section of a multi-chamber bioreactor. The cells are cultured on top of a membrane; (b) The fabricated multi-chamber bioreactor (from [89], reproduced with permission from Biotechnology & Bioengineering).

3.3.7. Liver

Liver is the main metabolic organ in an animal, and is significant for drug toxicity testing. Liver is also one of the most vascularized organs [92]. Microfluidic technology allows for the construction of microenvironments that closely model the in vivo liver system [93]. The most important aspects to be taken into consideration in designing liver on a chip are the cell-cell interaction through co-culture and the physical microenvironment.

For instance, liver specific functions of hepatocytes-fibroblasts co-culture on a 2D patterned surface are still significant after 6 weeks. In contrast, a conventional culture with pure hepatocyte will lose its phenotypic functions just after one week [24, 53] A 3D co-culture of hepatocytes and micro-vascular endothelial cells [MVECs] resulted in the formation of capillary structures after 8 days [25]. Co-cultures also improve the resolution and predictive values of drug clearance [26].

Microfabrication techniques have been used to construct an artificial liver sinusoid with endothelial-like barrier between the flow and the hepatocyte culture [54]. Such a barrier protects the cells from shear stress induced by the flow, while maintaining diffusion of nutrients and waste products [14]. Another way to mimic a sinusoid is using of a field-induced electrophoresis trap (Fig. 15) [54, 94]. Human liver cells (HepG2) were first positioned in a radial fashion. Human umbilical vein endothelial cells (HUVECs) are then trapped in the electric field. Fields above 2×10^3 V/m caused disruption of the cell membranes. This construct could be maintained in a flow rate of 20 μl/min. Other methods to position cells include direct cell writing and bio printing [95]. Syringes were used to deposit layer-by-layer cells, growth factors and scaffold material. Culturing a liver tissue slice has the advantage of closely resembling the complex organisation of the in-vivo system, as all cell types are present in their natural matrix. The disadvantage of tissue slices is the short lifetime of only up to 24 hours [96].

Cell used: Rat primary hepatocytes; Cryopreserved human hepatocytes and endothelial cells; HepG2 (human liver hepatocellular carcinoma); HepG2 /C3A (clonal derivative of Hep G2).

3.3.8. Lung

The lung is a very complex organ, with functions ranging from the molecular level (e.g. gas exchange) to the whole organ level (e.g. bulk airflow) [97]. This means, both molecular and macroscopic functions should be considered in designing a lung on a chip. During the normal respiration, cyclic stretching forces act on the lung alveolar epithelium and the adjacent endothelium, Fig. (16). Huh et al. [43] simulated cyclic stretching by culturing human alveolar epithelial cells and human pulmonary microvascular endothelial cells on opposite sides of a porous PDMS membrane. This work showed that the cyclic strain enhances the uptake of nanoparticles by the cells. In a subsequent work, this lung on a chip device mimicked drug toxicity responses of cancer patients treated with interleukin-2 (IL-2) and identified lead compounds that prevent the toxicity of IL-2 [98].

A key function of the lung is the exchange of oxygen and carbon dioxide. A surface facilitating gas transfer between the blood and the surrounding is needed for the lung model. Kniazeva et al. [99] fabricated an artificial lung in a micro-fluidic device consisting of multiple layers of branched micro-vascular networks. The device aims at the study of scaling effect associated with fluidic resistance and oxygen transfer [100]. Integrating a lung model, which includes a chamber mimicking the alveolus, into a more complex system of human on a chip requires the optimisation of the residence time inside the chamber and depends on the membrane used, Fig. (17) [101, 102]. Sun et al. [103] reported a more in-vivo like system that has an alveoli-like 3D-scaffold to study the migration of cancer cells in the lung. The scaffold consists of an array of uniform spherical pores in gelatine. The migration speed and direction of various lung cancer cell lines in this scaffold were measured. The results indicated that migration properties are different in a 2D and 3D environment, probably because of the different cell morphologies.
3.3.9. **Neuron**

Unlike most other cell types, neurons are elongated and highly branched. A neuron receives various signals via its dendrites and transmits them via a single axon (Fig. 18). Communication between neurons occurs at the synapses. Microfluidics technology allows for a high-resolution spatial control that enables the access to a single domain in the neuron network. Neurons extend their neuritis along the flow direction and topological features [104] and grow along polyelectrolyte adhesion line [105]. The type of surface coating determines the adhesion and morphogenesis of neurons. For instance, matrigel and lamine cause more neuritogenesis than poly-lysine [106]. Self-assembled adhesion of neurons is facilitated by coating the patterns with astrocytes. Again here, co-culture is the key for a longer culture time. For instance, hippocampal neurons and glia co-culture and culture of compartmentalised networks could be maintained for several weeks [107, 108].

Fig. (18). Neurons consist of dendrites and axons. Neurons are connected to each other via synapses at the end of the axons and dendrites.

Another advantage of microtechnology is the possible integration of electronic circuits on the neuron chip. The circuits must be confined to a specific location to establish contact and to pick up signals from a single neuron. One option is using “picket fences” to prevent the neuron from migrating away and thus losing contact with the electrodes, Fig. (19) [109]. Integrated electrodes were able to detect cell responses to external stimuli such as a serine hydrolase inhibitor [110]. Another option is using an integrated microelectrode array (MEA) or lateral patch-clamp to measure neuron activities [111, 112]. These detection methods enable high-throughput electrophysiology in neuron cultures.

**Cell used:** Neuronal cultures isolated from CD1 mouse embryos at E17; hippocampal neurons isolated from E18, E19 rats embryos; cortical neurons isolated from 18-day-old
Towards Human on a Chip: Recent Progress

Wistar rat embryos; RBL-1 (Rat basophilic leukaemia cell line); LUHMES (Lund human mesencephalic); SH-SY5Y (human neuroblastoma cell line); R28 (rat retinal cells); NG108-15 (neuroblastoma/glioma hybrid cell line: mouse neuroblastoma and rat glioma); N1E-115 (mouse neuroblastoma cell line); neurons isolated from the A-clusters in the central ganglion rings from the pond snail L. stagnalis.

3.3.10. Pancreas

The pancreas is essential for insulin production (Fig. 20). Together with the liver, intestines and fat, the pancreas controls the glucose level in the body. To date, research involving microfluidic pancreas models has focused on the islets of Langerhans, partly to measure their function before transplantation [113]. Of interest is the deterioration of the islet endothelium cell-morphology due to serum free media composition in a non-perfused system, Fig. (21) [114]. A fluid flow increased the density of endothelial cells, as the flow improves the penetration and therefore media exchange. Lee et al. [115] studied the insulin production in a pancreas model under a well-defined temporal glucose gradient. Adding glucose to the system stimulated the insulin production. Five minutes from the introduction of the glucose were needed for the islets to produce insulin. After 12 minutes, the insulin production decreases, similar to what happens in vivo [116].

Animal cells are used in these microfluidic devices, as they are more readily available. Only recently a human pancreatic β-cell line was used for secreting insulin. It took almost 30 years to get this human cell line because of the difficulty of obtaining a functional cell line that can preserve the characteristics of the parental cells [117].

Cell used: INS-1 (rat Insulin secreting beta cell); Islets of Langerhans from C57/BL6 mice.

3.3.11. Skin

Skin is the largest organ in the human body. The main function of the skin is to preserve the body homeostasis [118]. Cosmetic and drug companies are very interested in human skin models to test the toxicity of topical and transdermal products [119]. Although there was a media report in 2010 about testing skin allergies by replacing animals with a microfluidic chip which connects artificial skin with lymph nodes, an actual device has not been reported in scientific literature. Table 1 summarises the key achievements in the state of the art of organs on a chip discussed in the above sections.

4. CONNECTING ALL ORGANS TOGETHER: THE STATE OF THE ART

The various organs in the human body need to work together to maintain homeostasis. Extracellular fluid that circulated throughout the body is a key component in maintaining a stable internal environment. Just like the human body, micro-organs in separated compartments of a chip should be systematically connected to construct a human on a chip (HUC). A HUC consists of organ compartments linked through a microfluidic network mimicking the circulatory system of extracellular fluid [120]. A rational design of the microfluidic network in consideration of other organs is necessary [121]. The flow resistance within the compartments and their shape determine the required flow rate and the corresponding residence time. The size of the compartments determines the amount of cells. The device should reflect the organ mass ratio, blood distribution over the organs and the residence times as found in the human physiology. The residence time determines the duration of drug exposure, the drug uptake and its cytotoxicity. Such a system will not only improve the predictability of drug toxicity, but also improve insights to the mechanism of the drug lifetime within a physiological system.
Table 1. State of the art of organs on a chip.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Achievements</th>
<th>Authors, year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Modelling of the blood-brain barrier; Using micro slits or a membrane as</td>
<td>Prabhakarpandian et al. 2013; Yeon et al. 2012;</td>
</tr>
<tr>
<td></td>
<td>barrier; Tightness of the cell junctions is measured with TEER.</td>
<td>Griep et al. 2013</td>
</tr>
<tr>
<td>Breast</td>
<td>Migration of mammalian tumours into a 3D matrix.</td>
<td>Liu et al. 2009</td>
</tr>
<tr>
<td>Eye</td>
<td>Retinal cells seeded on PLGA scaffolds.</td>
<td>McUsic et al. 2012</td>
</tr>
<tr>
<td>Heart</td>
<td>A pump powered by cardio-myocytes; Real time measurement of the reactive</td>
<td>Tanaka et al. 2007; Park et al. 2007; Cheah et al.</td>
</tr>
<tr>
<td></td>
<td>oxygen species and cardiac contraction.</td>
<td>2010; Agarwal et al. 2013</td>
</tr>
<tr>
<td>Intestine</td>
<td>Cultivation of Caco-2 cells on a porous membrane, and exposed from two</td>
<td>Esch et al. 2012; Imura et al. 2009; Kimura et al.</td>
</tr>
<tr>
<td></td>
<td>sides with media; Simulating peristaltic bowel movement.</td>
<td>2008; Kim et al. 2012</td>
</tr>
<tr>
<td>Kidney</td>
<td>Shear stress and controlled the topology in vitro result in a tube-like</td>
<td>Frohlich et al. 2012; Jang and Suh 2010; Sciancaleporo et al. 2014; Gao et al. 2011; Jang and Suh 2010</td>
</tr>
<tr>
<td>Liver</td>
<td>Liver cells co-cultured with fibroblasts or micro-vascular endothelial cells,</td>
<td>Khetani and Bhatia 2008; Ukairo et al. 2013 [50];</td>
</tr>
<tr>
<td></td>
<td>Liver cells in chamber and a barrier between the flow and the hepatocyte</td>
<td>Sudo et al. 2009; Novik et al. 2009; Lee et al. 2007;</td>
</tr>
<tr>
<td></td>
<td>culture.</td>
<td>Toh et al. 2007</td>
</tr>
<tr>
<td></td>
<td>pulmonary microvascular endothelial cells on opposite sides of a porous</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDMS membrane; Lung was fabricated in microfluidics consisting of a multi-layer of branched micro-vascular network; Alveoli-like 3D-scaffolds to study cancer cell migration.</td>
<td></td>
</tr>
<tr>
<td>Neuron</td>
<td>Spatial control that enables the control of a single domain in the neuron</td>
<td>Takayama et al. 2012; (Reyes et al. 2004; Zeck and Fromherz 2001; Gebinoga et al. 2012; Morales et al. 2008; Tang et al. 2010</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Isle of Langerhans cultured in device and exposed to glucose to measure the</td>
<td>Lee et al. 2012; Wang et al. 2012</td>
</tr>
<tr>
<td>Skin</td>
<td>None</td>
<td><a href="http://m.technologyreview.com/biomedicine/24384/">http://m.technologyreview.com/biomedicine/24384/</a>)</td>
</tr>
</tbody>
</table>

Shuler’s group pioneered the HUC concept by showing the possibility of a human surrogate for predicting human response in clinical trials using a three-chamber (lung–liver–other organ) microscale cell culture analogue (µCCA) device with a PBPK model [122]. Viravaidya et al. [50] reported a similar device with the lung and liver compartments containing hepatocytes and lung epithelial cells of rat. A fat compartment was added as well, however, without cells. This device demonstrated that the metabolism of the drug Naphthalene resulted in a toxic component for liver cells. In another example, the response to the anticancer drug Tegafur (an oral pro-drug of 5-fluorouracil) in a 3D culture of colon cancer and hepatoma cells in the liver chamber and myeloblasts in the marrow chamber are similar to those of a clinical test. In contrast, a static test on 96-well plate showed no response [24].

Most recently, the same group demonstrated that the HUC system could be used to predict the toxicity of the anticancer drug 5-fluorouracil, with a pharmacokinetics–pharmacodynamics (PK–PD) modelling for three different dosages of the drug [123]. The results, analysed with a PK-PD model, showed a different response compared to that of a static cell culture. The authors suggested that in a lower concentration range of the drug the cells in the dynamic perfusion system were more sensitive than those in a static culture. Furthermore, the liver cells in both systems were more resistant towards the drug then the other cells, which is consistent with clinical findings. However, the differences in the drug effect on the different cell types were more pronounced in the dynamic system. This finding shows that response of the cell types is dependent on the environment they are in.

Focusing on a different aspect of the environment of a multi-cell culture, Yu’s group developed a multi-channel 3D microfluidic cell culture system with four compartmentalized microenvironments containing four different human cell types representing liver, lung, kidney and the adipose tissue for potential applications in human drug screening, Fig. (22) [123]. Each cell type actually needs its own specific micro-environment, be it physical or chemical cues. Interestingly, all the four types of cells were successfully grown with a common, serum-free culture medium which Yu’s group developed, while growth factors were locally released by gelatine microspheres [33]. This work showed that incorporating the spheres with the cells in every chamber could create cell specific microenvironments. The functions of the lung cells A549 were increased, while the other cells, including the liver cells C3A, were not affected. When C3A was directly exposed to TGF-β, the cell functions were inhibited.
Sonntag et al. [124] reported another multi-organ bioreactor that includes a liver, a brain cortex and a bone marrow compartments. However, the cells were keratinocytes (from the outer layer of the skin), fibroblasts and endothelial cells, which have no relation with the designed compartments. The device also has waste and nutrient reservoirs which are rechargeable and could sustain the culture for months. Unfortunately, the cultures have only been demonstrated for 14 days.

The latest development of HUC features an interconnected microfluidic hanging drop system [125]. Spheroid formations could be cultured in a hanging drop. The device consists of 4 columns with 6 hanging drops each. A microfluidic network interlinks all the hanging drops and allows for multi-tissue experiments, depending how they are connected. The drawback of this method is the exposure of the hanging droplet to its surrounding environment and evaporation.

5. PERSPECTIVE: INTEGRATION AND AUTOMATION

The current problem of microfluidic technology is the user friendliness. The introduction of pneumatic PDMS valves [126] was a big step forward computer-controlled microfluidics. However, a user interface and the integration with controllers are still lacking. In many applications, personnel with trained technical skills are still needed to operate these devices. As microfluidic devices become more complicated, a fully controlled system is desired. A good example for a fully automated system is the microfluidic Large Scale Integration (mLSI) system, which is comparable with integrated circuits in electronics [127]. Although on chip integration is progressing, it has not reached the same level of a robotic liquid handling system, which faced the same issues before.

The bottleneck in control is holding back the use of microfluidic devices for high-throughput and highly-complex analysis [82]. Ideally, an essay should be performed with just a press of a button, especially when dealing with cells, let alone multiple cell types. The system should be devised in such a way that all media, cells and drugs can be loaded automatically into the chip. Manual cell loading onto a chip still requires an extensive amount of training. The user needs to be familiar with the quirks of the chip as to not to lose or kill the cells. A recent trend is the integration of an automated system in an array format that potentially can work with conventional robotic sampling and loading [128].

Another bottleneck is the detection of the results. Often, the chip design needs to meet stringent optical requirement to work with conventional imaging facilities such as confocal scanning microscopy [129]. Sensors need to be integrated in to the cell culture chip. A major problem and but also a major benefit of microfluidics is the small quantity of products. For instance, the pancreatic β-cell produces 80 ng of insulin per one million cells [130]. However, only 5,000-10,000 cells are typically used in a chip. Standard analytical lab bench equipment would have to operate at their limits to detect these samples, introducing significant noise levels in the results. They also need a minimum volume of supernatant to operate. Furthermore, collecting samples and then transferring it to another piece of equipment invites contamination. Integrated and real-time detection should be made possible in the future development of HOC.

As a final point, automation and integration of the chip will result in better repeatability, as it does not depend on the user of the chip. To seamlessly connect with existing automated tools, microfluidics could be integrated into an existing robotic liquid handling system [131].

CONCLUSION

While an increasing amount work has been reported on 3D cell cultures and organ-on-a-chip, especially after a 2012 NIH/DARPA initiative injected US$76 million over for a period of 5 years in to this research area, there has been little progress made on human-on-a-chip devices. So far, only four research groups have attempted developing such devices.
One of the reasons might be the complexity issue. The first complexity problem lies in the device fabrication, if the organ sizes and residence times are to be considered. The other problem is culturing multiple cells on a single chip. Only Yu’s group has addressed the issue of different media for each cell type by mixing cells with gelatine microspheres loaded with specific growth factors in each chamber.

More efforts should be done to connect the organs on a chip together. The integration should consider the relative organ size, the residence time and the controlled microenvironments to establish an even more realistic in vitro human model. The human-on-a-chip could eliminate false drugs passing the animal test and approved by FDA, as shown by Shuler’s group, and have drugs that failed to pass the animal model tested again on an in vitro human model. This technology could lead to huge saving in drug development cost, shorter development time, less animal test, and even abolishing animal models.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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Towards Human on a Chip: Recent Progress


