5. Organ-On-A-Chip

A replacement to animal testing

Review Article

Shreya Mehta
Third Year Bachelor of Pharmacy
Department of Pharmaceutical Sciences & Technology

Krushali Powale
Third Year Bachelor of Pharmacy
Department of Pharmaceutical Sciences & Technology

Abstract

Here we describe an Organ-on-a-Chip (OC) which is a biomimetic microsystem. These are engineered microchips that can sum up the microarchitecture and functions of living organs, such as the lung, heart, artery etc. Each individual organ-on-chip is composed of a clear flexible polymer - about the size of a computer memory stick - that contains hollow microfluidic channels lined by living human cells. Because the micro devices are translucent, they provide a window into the inner-workings of human organs without having to invade a living body. There are envisions of creating the entire human body on chips. They might be useful for high-throughput analysis and screening of cellular responses to drugs, chemicals, particulates, toxins, pathogens, or other environment stimuli relevant to pharmaceutical, cosmetic, and environmental applications. Such a creation could spell an end to animal testing which is an expensive, ineffective and lethal process. It has the potential to revolutionize the whole drug testing process.

Key words: flexible polymer, microfluidic channels, animal testing.

1. Organ-On-A-Chip - an Overview

Microscale engineering technologies such as microfabrication and microfluidics, were first used to develop microchips. This enables extraordinary capabilities to control the cellular microenvironment with high spatiotemporal precision and to present cells with mechanical and biochemical signals. This has made it possible to microfabricate models of blood vessels, muscles, bones, airways, liver, brain, gut, and kidney. However, it has not yet been possible to engineer integrated microsystems that replicate the complex physiological functionality of living organs by incorporating multiple tissues,

including active vascular channels, and placing them in relevant organ-specific microenvironment.

Each individual organ-on-chip is composed of a clear flexible polymer - about the size of a computer memory stick that contains hollow microfluidic channels lined by living human cells. Because the microdevices are translucent, they provide a window into the inner-workings of human organs without having to invade a living body.

The researchers, now, seek to build 10 different human organs-on-chips, to link them together to more closely mimic whole body physiology, and to engineer an automated instrument that will control fluid flow and cell viability while permitting real-time analysis of complex biochemical functions. [1, 9, 10, 11]

2. Organs

2.1. Lung-on-a-Chip

Lung-on-a-chips are being designed in an effort to improve the physiological relevance of existing in vitro alveolar-capillary interface model which is the fundamental functional unit of the living lung.

It can be used for testing the effects of environmental toxins, absorption of aerosolized therapeutics, safety and efficacy of new drugs which may help accelerate pharmaceutical development by reducing the reliance on current costly models.

With every human breath, air enters the lungs, fills microscopic air sacs called alveoli, and transfers oxygen through a thin, flexible, permeable membrane of lung cells into the bloodstream. This lung-blood interface recognizes invaders such as inhaled bacteria or toxins and activates an immune response.

The lung-on-a-chip microdevice works by placing two layers of living tissues -- the lining of the lung's air sacs and the blood vessels that surround them -- across a thin (10µm) porous, membrane made of polydimethylsiloxane (PDMS).

The compartmentalization of the channels facilitates not only the flow of air as a fluid which delivers cells and nutrients to the apical surface of the epithelium, but also allows for pressure differences to exist between the middle and side channels. During normal inspiration in a human's respiratory cycle, intrapleural pressure decreases, triggering an expansion of the alveoli. As air is pulled into the lungs, alveolar epithelium and the coupled endothelium in the capillaries are stretched. Since a vacuum is connected to

the side channels, a decrease in pressure will cause the middle channel to expand, thus stretching the porous membrane and subsequently, the entire alveolar-capillary interface. The pressure-driven dynamic motion behind the stretching of the membrane, also described as a cyclic mechanical strain, significantly increases the rate of nanoparticle translocation across the porous membrane. (Fig 1)

In order to fully validate the biological accuracy of a device, the researchers inflicted injuries to the cells:

2.1.1 Pulmonary infection

The researchers tested its response to inhaled living E. coli bacteria. They introduced bacteria into the air channel on the lung side of the device and at the same time flowing white blood cells through the channel on the blood vessel side. The lung cells detected the bacteria and, through the porous membrane, activated the blood vessel cells, which in turn triggered an immune response that ultimately caused the white blood cells to move to the air chamber and destroy the bacteria.

2.1.2. Pulmonary inflammation

The researchers introduced a variety of nano-scaled particles into the air sac channel.

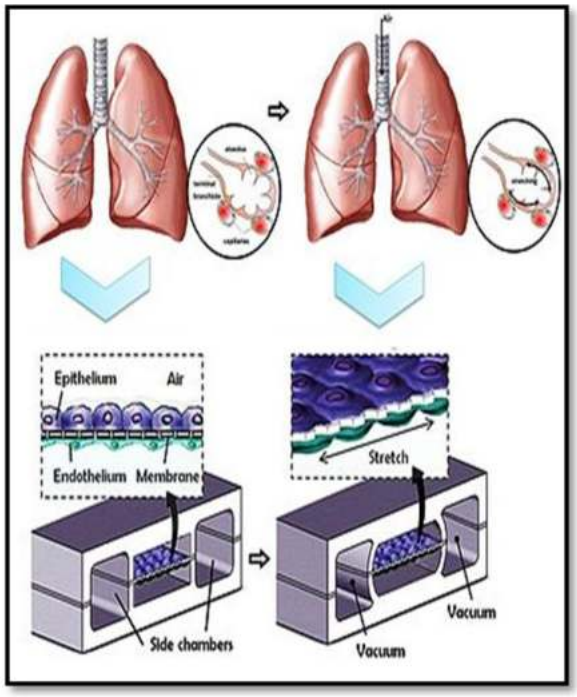

Figure 1: Lung-on-a-Chip [14]

The device consists of three hollow microchannels, and only the middle channel contains a horizontal porous membrane, coated on either side by either an endothelium or an epithelium tissue. The side channels are connected to a vacuum and can therefore simulate the stretching of the membrane. The contraction of the diaphragm triggers the intrapleural pressure to decrease, leading to an expansion of alveoli. This is the phenomenon essentially mimicked by this lung-on-a-chip.

Several types of these nanoparticles entered the lung cells and caused the cells to overproduce free radicals and to induce inflammation which is indicated by an increased production of epithelial cells and an early response release of cytokines.

A microfluidic lung-on-a-chip can more exactly reproduce the mechanical properties of a living human lung, its

physiological responses will be quicker and more accurate. [1, 9, 10, 12, 13, 14, 15]

2.2. Heart-on-chip

Traditionally, muscle physiology experiments require multiple tissue samples to obtain morphometric, electrophysiological, and contractility data. Furthermore, these experiments are commonly completed one at a time on cover slips of single cells, or in isolated muscle strips

Cardiomyocytes, are the cells that constitute heart which generate the electrical impulses that control the heart rate. "Heart on a chip" is a device that exploits muscular thin film technology – biohybrid constructs of an engineered, anisotropic ventricular myocardium on an elastomeric thin film – to measure contractility, combined with a quantification of action potential propagation, and cytoskeletal architecture in multiple tissues in the same experiment. The device was created using small thin strips of tissue made from heart muscle cells that are connected to electrodes to stimulate contraction.

The design and fabrication process of this particular microfluidic device entails first covering the edges of a glass surface with tape (or any protective film) such as to contour the substrate's desired shape. (See fig 2). A spin coat layer of PNIPA [poly(N-isopropylacrylamide)] is then applied. After its dissolution, the protective film is peeled away, resulting in a self-standing body of PNIPA. The final steps involve the spin coating of protective surface of PDMS over the cover slip and curing. These Muscular thin films (MTF) enable cardiac muscle monolayers to be engineered on a thin flexible substrate of PDMS. A micro contact printing technique was used to lay out a fibronectin "brick wall" pattern on the PDMS surface generated an anisotropic monolayer.

After the cutting of the thin films into two rows with rectangular teeth, and subsequent placement of the whole device in a bath, electrodes stimulate the contraction of the myocytes via a field-stimulation – thus curving the strips/teeth in the MTF. The contraction experiments were observed by looking vertically down onto the chip and monitoring the change in length as the strips contracted and bent up.

Observing the contraction response of the tissue allows scientists to study the effect of physiological factors or test drugs for cardiotoxicity. Replicating segments of heart tissue makes it possible to rapidly measure contraction data at the tissue level, rather than just studying individual cells. [2, 16, 17]

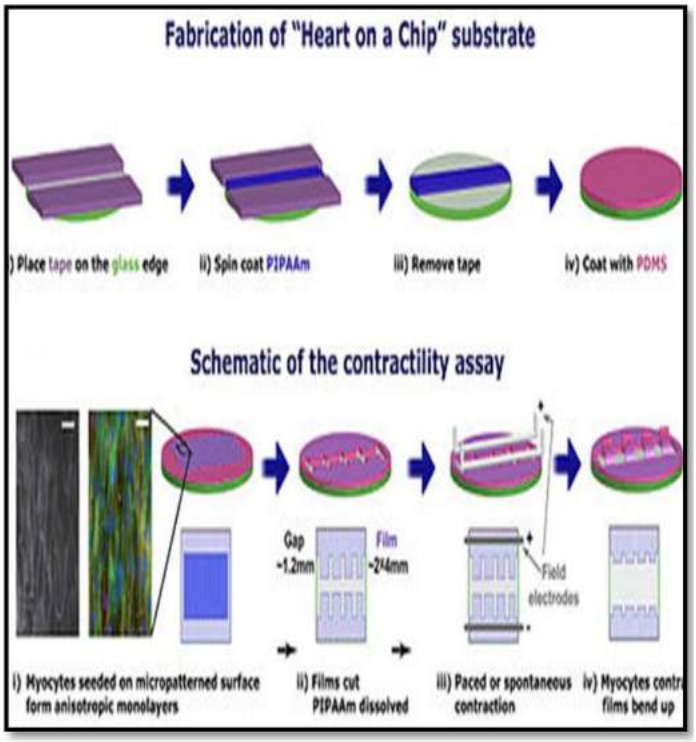

Figure 2: Preparation of the Heart-on-a-Chip Substrate and Contractility Test Samples[14]

After applying a stimulating the contraction of the myocytes via the field electrodes, strips/teeth in the MTF start to curl. Researchers have developed a correlation between tissue stress and the radius of curvature of the MTF strips during the contractile cycle, validating the demonstrated chip as a heart-on-a-chip.

2.3. Artery-on-a-chip

Scientists have developed a microfluidic platform on which fragile blood vessels can be fixed, so as to study the factors that promote and sustain various cardiovascular diseases. Microvascular structure and function are currently studied using two ways,

a. Isometric approach: where small arteries are mounted on two wires, or

b. Isobaric method: where arteries are drained and filled using glass micropipettes. Both of these procedures require manually skilled personnel and good laboratory facilities.

The platform involves loading and immobilising small arteries within a microfluidic channel where they can be maintained and analysed under physiological conditions that are very similar to those experienced in vivo. Forces within ranges that blood vessels experience in their natural environment can be explored without the use of mechanical tools. It acts as the basis of a microfluidic assembly line for complex structures from biological or colloidal building blocks.

Hallmarks of various cardiovascular diseases involve pathologic change of structure and function of small blood vessels, which can be well analyzed, using these chips. This microfluidic device can be used for the assessment of resistance artery structure and function under physiological conditions (37 °C, 45 mmHg transmural pressure). This device could be used to routinely screen drug candidates on viable arteries, potentially speeding up the drug development process and reducing the need for animal experimentation.

Dose effects were checked in mouse artery, of up to ten dose–response sequences of intact mouse mesenteric artery segments (diameter ≈ 250 micrometres and length ≈ 1.5 mm) in a well-defined microenvironment. The application of phenylephrine or acetylcholine (homogeneous condition) yielded dose–response relationships that were identical to conventional myography techniques. Following is the dose response curve seen in mouse mesenteric artery chip on application of phenyleprine.

The microfluidic platform allows us to address new fundamental biological questions, replaces a manually demanding procedure with a scalable approach and may enable organ-based screens to be routinely performed during drug development. [3, 4, 7, 8]

2.4. Kidney on a chip

A kidney-on-a-chip device has the potential to accelerate research encompassing artificial replacement for lost kidney function. Generally, dialysis patients go to a clinic up to three times per week. Artificial kidney research is striving to bring transportability, wearability and perhaps implantation capability to the devices through innovative disciplines: microfluidics, miniaturization and nanotechnology.

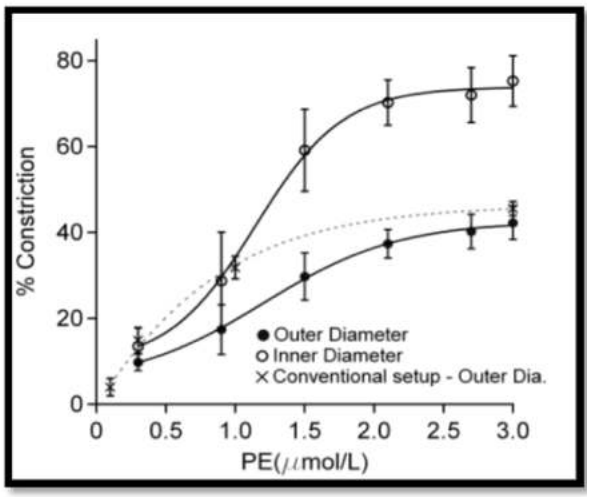

Figure 3: Phenylephrine Dose Response Consistent with Conventional Data

2.4.1. Example: Nephron-on-a-Chip

The nephron is the functional unit of the kidney and is composed of a glomerulus and tubular structures. Nephron on chip is a bioartificial device that replicates the function of the glomerulus, PCT (proximal convoluted tubule), DCT (distal convoluted tubule) and Henle's loop.

Renal tubules are involved in filtering the blood and producing urine. They are important to study as many drugs are secreted into the kidneys via these cells. These cells play roles in many kidney diseases, which are often due to the tubules being damaged or reacting to various molecules.

Each part of the device has its unique design, generally consisting of two microfabricated layers separated by a membrane. The only inlet to the

microfluidic device is designed for the entering blood sample. In the glomerulus' section, the membrane allows certain blood particles through its wall of capillary cells, composed by the endothelium, basement membrane and the epithelial podocytes. The fluid that is filtered from the capillary blood into Bowman's space is called filtrate or primary urine.

In the tubules, substances are added to the filtrate for urine formation, and some substances reabsorbed out of the filtrate and back into the blood. In the PCT, complete absorption of nutritionally important substances takes place. In the device, this section is a straight channel, but blood particles going to the filtrate have to cross the previously mentioned membrane and a layer of renal proximal tubule cells. The second segment of the tubules is the loop of Henle where the reabsorption of water and ions from the urine takes place. The device's looping channels strives to simulate the countercurrent mechanism of the loop of Henle. Likewise, the loop of Henle requires a number of different cell types because each cell type has distinct transport properties and characteristics. These include the descending limb cells, thin ascending limb cells, thick ascending limb cells, cortical collecting duct cells and medullary collecting duct cells.

The micro-model of kidney physiology will also feature two parallel structures – small blood vessels and the surface lining of the renal tubules. This aspect of the device will enable researchers to study the complex interactions between these two structures, which are normally in intimate association inside each of the functional units of the kidney, the nephrons.

One step towards validating the microfluidic device's simulation of the full filtration and reabsorption behavior would include demonstrating that the transport properties between blood and filtrate are identical with regards to where they occur and what is being let in by the membrane. For example, the large majority of passive transport of water occurs in the proximal tubule and the descending thin limb, or the active transport of NaCl largely occurs in the proximal tubule and the thick ascending limb. The device's design requirements would require the filtration fraction in the glomerulus to vary between 15%-20%, or the filtration reabsorption in the proximal convoluted tubule to vary between 65%-70%, and finally the urea concentration in urine (collected at one of the two outlets of the device) to vary between 200-400mM.

However, the flow of blood and urine means that renal tubular cells are exposed to shear stresses. It also brings challenges

to making physiologically relevant models using the cells. To counter this scientists developed a multilayer microfluidic device and optimised the growth conditions for the renal tubular cells. The cells are grown on a permeable support that is placed over a well containing outer tubular fluid (playing the role of blood) whilst a continuous stream of inner tubular fluid (precursor urine mimic) is passed over the cells. This leads to the cells growing and functioning as they would in the body. This device was able to show that hormone stimulation causes a water-transporting protein to move within the cells. The model even mimics how the cells respond in vivo, helping in understanding of cellular mechanisms of disease. [5, 6]

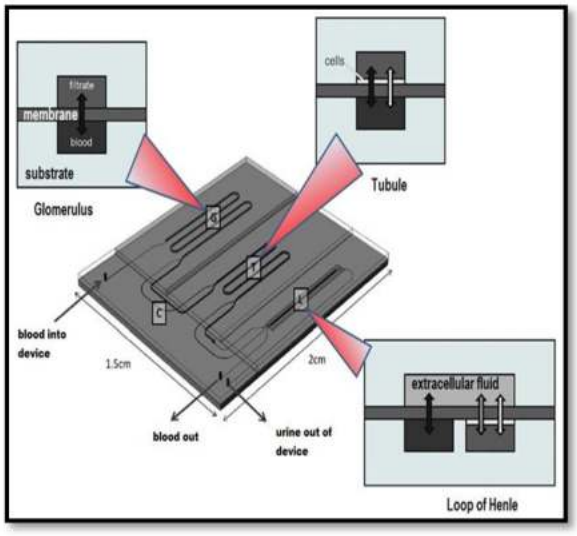

Figure 4: Schematic of a Nephron-on-a-Chip Device with Cross-Sections of 3 functional Units – C – Connector; G – Glomerulus;T – Tubule; L – Loop of Henle / Black arrows: passive transport / White arrows: cell-mediated active transport. [14]

Advantages

With a complete human imitating system, scientists can see the biochemical effects of drugs across the entire human body.

- The concept replicates the function and composition of an organ on a chip that can be easily tested in order to study effects of diseases, toxins, and pharmaceuticals.
- It creates a versatile platform capable of accurately predicting drug and vaccine efficacy, toxicity, and pharmacokinetics in preclinical testing.
- 3D on the chip – allows scientists to assess the effects of a candidate drug on gene expression, on proteins in the cardiovascular system, the neurological system, and more.

Disadvantages

- Are not scalable due to limited the number of laboratories carrying out essential microvascular research.
- Expensive and will take ample amount of time to be put in practice.

3. Conclusion

The goal is to develop human tissue chips that simulate the structure and function of human organs, such the lung, heart, liver, and kidneys. Scientists could then use

these tissue chips to test drug candidates and predict their safety before the next step, human drug studies. This approach is expected be more rapid and cost effective than those currently available. The National Institute of Health, pointed to studies that show that more than 30 percent of promising medications have failed in human clinical trials because the drugs were found to be toxic, despite pre-clinical studies in animal models. Tissue chips may offer more accurate predictions of the side effects of potential therapeutic agents because they contain human cells. These bioengineered devices will produce relevant physiological functions and will reflect the complexity and diversity of living organs, including genetic differences, disease complexity and pharmacological responses.

References

1. Dongeun Huh, Benjamin D. Matthews, et al."Reconstituting Organ-Level Lung Functions on a Chip", Science, **328** (5986) (2010) 1662-1668.
2. Anna Grasberg, Patrick W. Alford et al. "Ensembles of engineered cardiac tissues for physiological and pharmacological study: Heart on a chip" Lab on chip, **24** (2011).
3. Kyung-Jin Jang1,et al., Wyss Institute for Biologically Inspired Engineering at Harvard University, USA
4. Seoul National University, Korea; and Children's Hospital, Harvard Medical School and Harvard School of Engineering and Applied Sciences, USA July 27, 2012.
5. By Leila Gray UW Health Sciences/UW Medicine. Seattle researchers to engineer kidney tissue chip for predicting drug safety
6. Sarah C.P. Williams, The Wyss Institute, Multiple organ-on-chips into a single automated instrument that tests whole-body effects of drugs. 07/25/2012.
7. Axel Günther, Sanjesh Yasotharan, et al. Lab Chip,(2010), 2341-2349 Received 25 Mar (2010), Accepted 14 Jun (2010) First published on the web 06 Jul 2010 .
8. Artery-on-a-Chip Symposium and Workshop 2011 March 8, 2011 – The Faculty Club, Main Floor Dining Room, University of Toronto, 41 Willcocks Street Toronto, Ontario M5S 1C7
9. Dongeun Huh, Daniel C. Leslie, Benjamin D. Matthews, Jacob P. Fraser1, Samuel Jurek, Geraldine A. Hamilton1, Kevin S.et al "A Human Disease Model of Drug Toxicity–Induced Pulmonary Edema in a Lung-on-a-Chip Microdevice" SciTransl Med 7 November (2012): Vol. **4**, Issue 159, p. 159ra147
10. Robert F. Service, "Lung-on-a-Chip Breathes New Life Into Drug

Discovery"Science 9 November 2012: Vol. 338 no. 6108 p. 731

11. "Human-on-a-chip may end lab animal testing" Mumbai mirror, tech talk, Dec 2012.
12. Wyss institute home page http://wyss.harvard.edu/viewpressrelease/36/living-breathing-human-lungonachip-a-potential-drugtesting-alternative (Accessed Dec 2012)
13. Red orbit-universe online http://www.redorbit.com/news/health/1112727640/disease-organ-on-a-chip-model-110812/(Accessed Dec 2012)
14. http://en.wikipedia.org/wiki/Organ-on-a-chip. (Accessed Dec 2012)
15. DrakeBennett, "Making Human Organs on a Chip" Bloomberg business week, June 27, 2012.
16. Med gadget home page http://www.medgadget.com/2011/11/beating-heart-on-a-chip-may-revolutionize-in-vitro-cardiac-studies.html (Accessed Dec 2012)
17. RSC-advancing chemical sciences http://www.rsc.org/chemistryworld/News/2011/November/10111102.asp(AccessedDec 2012).