

Review Article

Organs-on-Chips : Innovation to new testing technology research and discovery

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ABSTRACT

Since the last few years, many recent advances in biomedical research are related to the combination of biology and micro engineering. Micro engineering now enables the incorporation of small devices into 3-D culture models to reproduce the complex micro-environment of the modelled organ, often referred to as organs-on-a-chip (OoCs). This review describes history of organ chip, application and various OoCs developed to mimic liver, brain, kidney, lung tissues.

1. INTRODUCTION

The recently introduced field of organs-on-chips has the potential to address these new discoveries. These organ-on-chip models, which contain micrometer-sized, fluid-filled channels in which human cells can be cultured, provide opportunities for engineering controlled culture environment that resembles the microenvironment of a certain organ by tuning mechanical, biochemical and geometrical aspects [1]. There are many recent new advanced technology and research in biology and micro-engineering or in combination. More physiological behaviour is expected from such a combination of cells and engineering, resulting in often more précised predictive value. Previously preclinical drug testing methods can be done on animal models to predict pharmacology and toxicology profiles, but these data does not gives accurate results related to pharmacodynamic and pharmacokinetic profile. Because of these all limitations to overcome these problem researchers has been develop new device such as organ-a-chip. It is device which resembles to cell structure and have all characteristics maintained at human physiology. It has many advantages such as, it improve the pharmacodynamic and pharmacokinetic data, it also gives most accurate and specific results (specific results in terms of targeted drug delivery system). It has most important benefit as it is saving

life of animals, and prevent them from pain full methods. It is also not a time consuming method.

Organ -a-chip, it is micro-fluidic device which generally provide standard controllable microenvironment for living cell in micrometer sized chamber for maintaining physiology of cell, tissue or organ. Organ-a-chip is related to *in-vitro* study. These technology also being used in all main human body organ, such as lungs, skin ,bone marrow, heart, gut, liver, pancreas, kidney, eye even system that mimics the blood brain barrier.

2. HISTORY

Donald Ingber has a record of starting scientific revolutions. He was the first to realise that cells sense and respond to their physical environment as respect to an internal network of fibres, similar to the function of the human physiology. His team was also the first to marry electronics to biology to make living “organs on chips” – tiny automated bits of human organs that promise to replace the new combination of technology for the ease of drug discovery. Ingber said that scientists typically test potential pharmaceuticals on animals, but more often the predictions from animals fail when a compound is tested in humans.

Performing initial tests on people, of course, is too dangerous. The proposed solution is to do studies with human cells, but not just cells in a dish—cells that exhibit organ like structures and functions. A human organ -chip is a device that integrates one or several laboratory functions on a single chip that deals with handling particles in hollow microfluidic channels. That has been developed from previous few years. Basic advantages if is in handling particles at such a small scale include lowering fluid volume consumption (lower reagents costs, less waste), increasing portability of the devices, increasing process control (due to quicker thermo-chemical reactions) and decreasing fabrication costs. Simultaneously, there is virtually no mixing between any streams in one hollow channel. Where as in cellular biology, this rare property in fluids has been encouraged to study complex cell behaviours e.g. cell motility in response to chemotactic stimuli, stem cell differentiation, axon guidance, sub-cellular propagation of biochemical signalling and embryonic development [2].

3. HUMAN ORGANIC CHIP

Now a days *in-vitro* preclinical testing can be done on 2D and simple 3D culture system. Two dimensional culture systems is simple technique, widely used because it is cost effective models used to study toxicology effect of drug with proper maintenance. The physiological and pharmacokinetic parameter can be studied. In principle, 3-D culture systems can be described as the assembly of primary cells, cell lines, or stem cells into a 3-D structure using specific cell culture plates. The spatial organization within the 3-D culture systems allows the cells to exhibit cell–cell interactions and the build up of an extracellular matrix, which consequently provides a physiological microenvironment where cells are subjected to polarization and ionization [3]. 3-D models are currently available and new ones are increasingly being developed to model almost all the organs of the human body.

Organ –on –chip is generally composed of clear flexible polymer which has standard size of computer memory stick that contains hollow micro–fluidic channels which are connected to human cell interfaced with a human endothelial cell –lined artificial vasculator. Mechanical force can be applied to mimic or stagnant the physical microenvironment of living organ including breathing motion in lungs and peristalsis like deformation in the intestine. Organ on chip generally integrated in combination with micro–engineering and biology, in which an electronic unit is connected to chip, supported to the redevelopment of micro–environmental by some mechanical stress or support. Some chips are developed by simply integrating a peristaltic pump, while others integrate various sensors that can simultaneously and continuously record the pH and oxygen consumption, monitor the morphology, and detect soluble proteins released into the culture medium [4].

Organ-on-chip are created from micro-engineering as follows:

- Recreate tissue-tissue interface (e.g analyze transport, absorption, transport, permeability, conductivity)

- Provide mechanical clues necessary for relevant physiology (e.g fluid flow, cyclical mechanical strain, air-liquid interface, directional clearance)
- Precisely orient cells for high-resolution real-time imaging (e.g enable analysis of molecular and cellular mechanisms at critical tissue boundaries)
- Place cells in separate channels to study different cell populations (e.g harvest cells or medium independently for molecular genetic analysis sample oriented (e.g., luminal) secretions in real-time)
- Control fluid flow through microfluidic channels (e.g supports long-term culture, enable pharmacokinetic analysis, permits co-culture of microbiome)
- Create endothelium-lined vascular channels (e.g permit real-time analysis of recruitment of circulating immune cells, It potentially can use of blood or plasma to feed organ chips. This enables physiological vascular coupling between different organ chips).

Below mentioned is an example of Organ-on-chip as an electronic unit integrated into the chip supports the generation of the physiological microenvironment, for example, directing a flow of medium or applying pressure on a membrane to cause a mechanical stress (Figure 1).

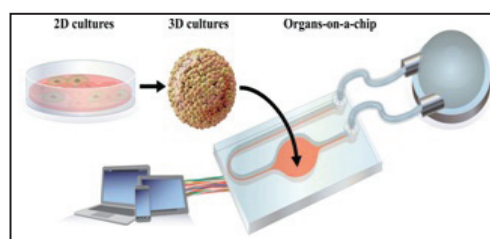


Fig. 1. *In-vitro* models of simple monolayer culture Organ-on-chips

4. BASIC WORKING MECHANISM OF ORGAN-ON-CHIP (OOC) DEVICES.

Organ-on-chip systems are basically elaborated micro-engineered physiological systems that reconstitute the key features of specific human tissues and organs and their interactions as depicted in Figure 2.

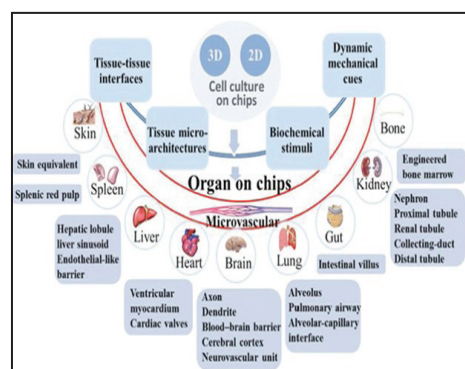


Fig. 2. Representation of organ on chip device and concept of modelling, a complex microenvironment and their existing simulation of functional units

Key point in OOC designing includes the following:

The next step is application of micro-engineering fundamentals to introduce the key aspects into a working organ specific model. Now, the cells are introduced into the model under designed stimuli and they self organize themselves based on the stimuli and display comparatively more realistic function than the conventional 2D *in-vitro* models. The final step is to measure the functional output parameters of the cultured cells.

Earlier, with 2D and 3D cell cultures, efforts were taken to control and regulate the cell growth, shape and other cellular events but due to lack of precise 3D environment, these models suffered with inaccuracy and reliability in recapitulating the issue and organ specific systems [8]

But with the state of art OOC technology, new possibilities to create efficient *in-vitro* models with organ specific microenvironments, tissue micro-architecture reconstruction, spatio temporal chemical gradients, tissue specific interfaces, crucial dynamic mechanical clues and biochemical signals have emerged. In this section, we describe recent progress in this field and currently reported OOC devices such as liver, kidney, intestine, kidney, heart, skin and blood vessels.

Can organs-on-chips replace drug testing on animals?

- In his address at SLAS2015, Dr. Donald Ingber told the about demerits of biological testing methods:
- Testing a single compound can cost more than \$2 million
- Cells cultured in dishes don't function as they do in our bodies
- Animal studies take years to complete
- Innumerable animal lives are lost
- Results often don't predict clinical responses
- Lack of new drugs reaching patients

About 85% of therapies fail in early clinical trials and of those that make it to Phase III, generally the last step before regulatory approval, only half are actually approved. "Less drugs are getting to patients, more companies are getting frustrated and it's clear that we need better lab models that mimic whole organ function. Organs-on-chips devices are of the size of a computer memory stick that contain human cells immersed in the blood vessels and tissue that normally form living organs [9]. Because the environment of each organ chip is so similar to that of the human body, it actually functions the way the human organ does. The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) performed a systematic survey of 271 animal studies and found that only 32 (12%) reported using random allocation to treatment or control and that investigators were blinded to the allocation in only 14% (5/35) of studies that used qualitative scoring [10].

5. APPLICATION OF HUMAN ORGAN-ON-CHIP

5.1. Diagnostic applications

It is used for testing in the field of biomedical sciences. The complex live systems and richness of biological processes are

stimulating factors for new LOC approaches, and these emerging technologies are gradually changing the scenario, and hence, we can get experimental solution at the molecular level.

5.1.1. On-chip DNA hybridization

An on chip deoxyribonucleic acid (DNA) hybridization assay refers to the bioassay conducted on the microfluidic system/device based on the nucleic acid hybridization technique. From its earlier applications in 1980s, it has been evolved as a powerful tool to detect and identify the presence of a specific DNA sequence. On-chip DNA hybridization systems are amalgamation of advantages of both microfluidics and hybridization [11].

There are two types of DNA Chips:

- 2-Dimensional DNA Chip (on-chip) -> DNA Microarray
- 3-Dimensional DNA Chip (in-chip) -> Lab-on-Chip

DNA Chips are used for clinical diagnosis of genetic diseases, cancer diagnosis & prognosis, detection of viruses & bacteria, drug-resistant microbial typing, environmental monitoring, detection of pathogens/carcinogens, food safety & processing, drug discovery, simultaneous analysis of thousands of genes, screening of drug molecules, biomedical research, differential gene display/mutation detection, discovery of new genes and functional genomic study [12].

5.1.2. PCR device

PCR device consists of the following four major parts: a disposable chamber chip with microchannels and pumping membranes, a heater chip with microheaters and temperature sensors, a linear array of electromagnetic actuators and a control/sensing circuit. Apart from the small size ($67 \times 67 \times 25$ mm³) and less power consumption (5V DC) and reduced volume of DNA solution, this system could effectively reduce the PCR process time into one-third of the time required by typical commercial PCR system [13]. In another approach, came forward with their KRas mutation detection on chip. Figure 2 shows schematic of the on-chip detection device. They aimed to develop a fast and reliable chip-based K-Ras mutation based on existing microfluidic chip platform for visual signal readout of K-Ras mutation profiling.

5.2. Drug delivery applications

The major objective of drug delivery systems is to localize the pharmacological activity of the drug at the site of action as targeted drug delivery systems directly deliver to the desired site of action with minimum interaction with normal cells. This phenomenon is especially important for anticancer drugs, as their toxicity to healthy cells is a cause of concern to improve therapeutic response and patient compliance. Microfluidic technology gives control over particle size, composition, encapsulation rate and better performance of nanoformulations, which have a great impact on the cancer survival rate.

5.3 Microarrays technologies

A microarray is an analytical device that comprises an array of molecules (oligonucleotides, cDNAs, clones, PCR products, polypeptides, antibodies and others) or tissue sections immobilized at discrete ordered [14]. In a general microarray device, sample solutions are confined in microfabricated channels and flow through the probe microarray area. Enhanced sensitivity is obtained due to high surface-to-volume ratio in microchannels of nanoliter volume and advantages of both fields can be exploited simultaneously by combining DNA microarray with microfluidics. Consumption of small volumes in microfluidic systems is an added advantage to develop low-cost, compact and portable LOC systems. Secondly, the surface hybridization of target DNA can also be accelerated on microfluidics platform by electrokinetic delivery of negative charged DNA molecules on to the probe area [15]. Many companies are involved in designing microfluidic technology for various high-throughput applications, such as immunoassays, diagnostic devices, single molecule DNA and protein detection as well.

6. RECENT DEVELOPMENT ON HUMAN ORGAN ON CHIP

6.1. Liver-On-Chip

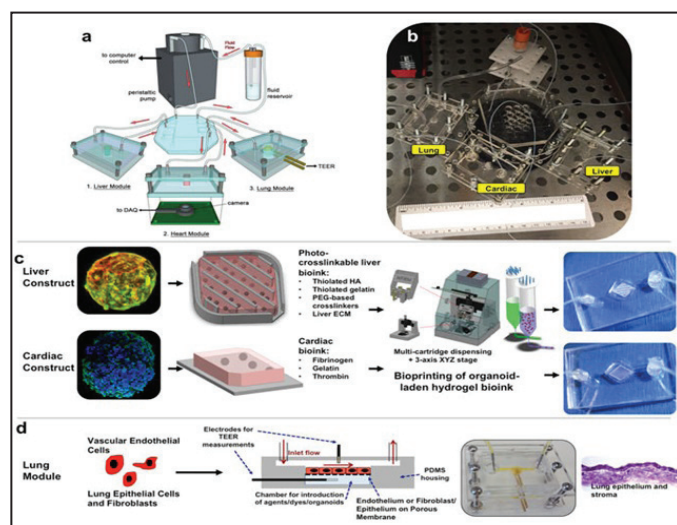


Fig. 3. Human organ-on-chip (a) Chip module for flow of fluid. (b) Types of organ on chip. (c) Liver and cardiac construct module. (d) Lung module

In human body liver is the major metabolism organ and its function include regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It is second largest organ in human body. Xenobiotic compound are metabolized in liver and that emphasizes the need of liver culture system because a is physiological relevant xenobiotic drug are absorbed, then it goes into the blood circulation after that it reaches to the liver and where they undergo two-step process, to transform a generally lipophilic compound into a more hydrophilic molecule.

In first pass metabolism (phase-1) drugs undergo oxidation, reduction, and hydroxylation mainly catalysed by enzyme cytochrome p-450. In phase II metabolism they generally undergo glucuronidation, sulfation and acetylation reactions, which is mainly catalysed by transferases enzyme.

Liver biochip should contain nutrient zonation which reproduces the physiological metabolism of drugs. Such a gradient of nutrients can be implemented *in-vitro* by forming liver spheroids. In the spheroid model, cells located near the core have limited access to nutrients compared with the cells located more peripherally. Liver is the highly perfused organ in human body and which is generally act as bioreactor to metabolise the compound and then transform into the blood circulation at high rate, metabolic waste product excreted by hepatocytes function. Now a days many liver-on-a-chip systems are available, which can be attributed to the following:

- (a) The liver is an important organ for drug metabolism,
- (b) Recent improvements in the development of 3-D liver models
- (c) The relatively simple requirement for creating the desired microenvironment (i.e. a simple medium flow).

Recently Bhise et al have developed a liver-on-a-chip platform using bio-printed HepG2/C3A spheroids as a 3-D liver model, which contains PDMS chip has three chambers: a central bioreactor, containing the spheroids and two external chambers connected to the inlet/outlet of a syringe pump. By using PDMS Bavli et al. used a similar system to create a liver-on-a-chip, in which micro-wells covered with a glass layer and connected to a pump. There are incorporated sensors in the plate that were combined with oxygen-reactive micro-particles embedded in the tissues to measure the levels of glucose and lactate as well as monitor the liver tissues in real-time. By using an off-chip sensor unit, glucose consumption and lactate release were measured every 20 min.

Application of liver on chip

- (a) **Detection of alcoholic liver disease:** Liver spheroids are exposure (48h) at different concentration of ethanol and followed by a three day recovery. Exposure spheroids shows result such as impaired viability, morphology, and albumin secretion compared with control tissues.
- (b) **Identification of metabolites-** After metabolism, identification secondary toxic metabolite, as well as identification of main metabolite of compound and potentially low concentration of compound also identified.
- (c) **Liver cancer testing:** Generally HepG2 and HepC3 cell line used as liver cell alternate, generally HepC3 cells are metabolically more active than the HepG2. Recently newly developed HepaRG™ cell line which has superior characteristics than above two. HepaRG™ cells are more sensitive to hepatotoxicagents than the primary human hepatocytes.

6.2 Multi-Organ-Chip

Microscale devices that mimic human organs could provide a much more realistic environment for drug discovery. Multi-organ-on-a chip (MOC) systems mimic organ interactions observed in the human body and aim to provide the features of compound uptake, metabolism and excretion; while simultaneously allowing for insights into biological effects. MOCs might represent a new paradigm in drug development, providing a better understanding of dose responses and mechanisms of toxicity, enabling the detection of drug resistance and supporting the evaluation of pharmacokinetic–pharmacodynamics parameters. Nevertheless, building valid artificial organs requires not only a precise cellular manipulation, but a need an understanding of the human body’s fundamental intricate response to body with external environment. A common concern with organs-on-chips lies in the isolation of organs during testing. The overall complexity of the chip can be tailored depending on the need. Some chips are developed by simply integrating a peristaltic pump, while others integrate various sensors that can simultaneously and continuously record the pH and oxygen consumption, monitor the morphology, and detect soluble proteins released into the culture medium.

Figure 3 shows the module of the human organic chip and its micro-fluid components that include five major parts such as isolating, sorting, viability, differentiate monitoring and migration [5]. These are the step or module for proper understanding of the working on the chip at the lab scale and guide us well to obtain the precise result or its physiological or physiochemical effect. Typical advantages are:

- Low fluid volumes consumption (less waste, lower reagents costs and less required sample volumes for diagnostics)
- Faster analysis and response times due to short diffusion distances, fast heating, high surface to volume ratios, small heat capacities.
- Better process control because of a faster response of the system (e.g. thermal control for exothermic chemical reactions)
- Compactness of the systems due to integration of much functionality and small volumes
- Massive parallelization due to compactness, which allows high-throughput analysis
- Lower fabrication costs, allowing cost-effective disposable chips, fabricated in mass production [6]
- Part quality may be verified automatically.
- Safer platform for chemical, radioactive or biological studies because of integration of functionality, smaller fluid volumes and stored energies [7].

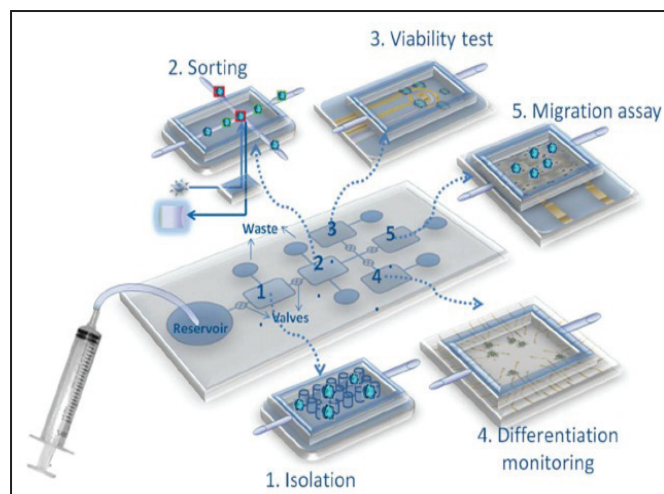


Fig. 4. Module of chip and its multi fluid components

While useful for the toxicological testing of single compounds, the MOCs discussed above do not allow for high throughput experiments. MOC system composed of single chambers each chamber possesses an inlet and outlet to allow for a syringe pump to create a flow of medium. The chambers can have different designs to accommodate different types of cultures. The idea behind the system is that each chamber can be either isolated, to allow, the culture to settle and mature, or can be connected to other chambers using a simple connector that creates a bridge between the chambers. With the connector and the flow created by the syringe pump, the two chambers can exchange medium and metabolites [16, 17].

These MOCs all connect two or more tissues using microchannels. As the cell culture medium in these MOC systems has to cover all organ cultures simultaneously, by finding an appropriate medium may prove difficult. Even if only one organ culture does not draw sufficient nutrients from the chosen medium, the entire MOC may be “under testing.” As per future developments of MOC platforms would include blood or a blood surrogate that supports the growth and health of all the organs [18].

6.3 Heart-On-Chip

As we know cardiovascular diseases (CVDs) are the first cause of death globally. It is estimated that 17.5 million people died from CVDs in previous years, representing 31% of global death. Due to many problems such as life style, increasing in fatty substances e.g. cholesterol, heart attack is the major problem. In a mammalian body, in the circulatory system, heart is responsible for pumping blood, and has a most important feature of auto rhythmicity, modulated by the endocrine and nervous systems. Auto rhythmic heartbeat leads to heart rate fluctuant mechanical stress at the endothelial cell (EC) surface, which is important for vascular phenomena such as vessel remodelling, endothelial permeability, vasoregulation, blood formation, and vascular pathology, including platelet thrombus formation and atheroma. With the help of microfluidic chip technology, replicating functional cardiovascular organ model *in-vitro* cardiovascular functions can be tested with the help of Organ-on-chip devices.

6.4 Kidney-On-Chip

Kidney has essential function of filtration, re-absorption, and secretion and they are work by simple mechanism and maintains the ionic balance of human body. Renal model is important to understand the mechanism of nephrotoxicity and to decrease the high instances of drug-induced nephrotoxicity [19]. Fluid shear stress is responsible for renal cell gene expression along the tubule, linked to apical/basal polarization or cytoskeleton reorganization where *in-vitro* renal cell polarization can be done on porous membranes as substrates for cells to grow on and to induce a mild apical/basal polarization. Improvement of kidney models are based on *in-vivo* observation that renal cell are exposed to blood flow. Kidneys on-a-chip designed so far have, in addition to a porous membrane, an apical compartment for medium circulation.

By using these concept, Jang and Suh developed the inner medullary collecting duct cells subjected to shear stress displaying a strong apical/basal polarization, which is based on the

expression of aquaporin 2 and the Na⁺, K⁺ pump. At the cell edges, increase localization of adherens junction proteins and cytoskeleton affect the flow of medium. Cells maintained in the chip are more affected in a static condition than by the hydrogen peroxide exposure [20].

Applications of kidney-on-a-chip systems are not only limited to toxicological assessments. They include new treatments for various nephropathies and calcium monitoring. Calcium monitoring uses human proximal tubular cells combined with a PDMS micro-fluidic device to mimic calcium phosphate stone formation in kidneys. Variation in the tubular flow speed could initiate some nephropathies. This mechanism was studied *in vitro* by Rydholm et al. using a chip made out of either PMMA or silicon glass and with several crossing channels to produce a controlled laminar flow across MDCK cells. Calcium variations within the cells were monitored to show that variations in flow speed can change the kinetics of cellular calcium responses. Kidney-on-chip might also be useful in identifying drugs that may cause nephrotoxicity, in understanding their mechanism of action and in finding biomarkers to accurately predict renal toxicity.[21]

6.5 Lung-On-Chip

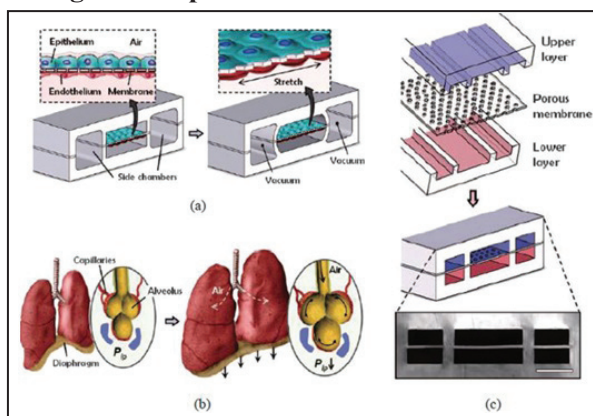


Fig. 5. Schematic of lung on-chip system.

Lung-on-chip is the microreplica of the lung on a microchip. This is used for nanotoxicology studies of various nanoparticles that are introduced into the air channels and to understand the pulmonary diseases where due to the formation of liquid plug that blocks small airways and obstruct gas flow in alveoli [22]. To understand the mechanism of liquid plug propagation and rupture, Huh et al. designed a microengineered system that consists of two PDMS chambers separated by thin polyester membrane with 400 nm pores. This system mimicked an *in-vivo* basement membrane for small airway epithelial cells attachment and growth. These inventions reconstituted the critical lung functions and can be applied for *in-vivo* models in environmental toxins, absorption of aerosolized therapeutics and the safety and efficacy of new drugs. Such a tool may help accelerate pharmaceutical development by reducing the reliance on current models.

7. CONCLUSION

As we know Drug discovery and research is the prime aspect of any pharmaceutical company. The past 50–60 years have witnessed significant scientific and technological growth in entire field of biotechnology, computational drug design & screening and advances in scientific knowledge, such as an understanding of disease mechanisms, new drug targets and biomarkers discovery. Hence these devices are becoming more and more common in research centers, clinics, and hospitals, and are contributing to more accurate studies and therapies, making them a stable technology for future drug design. The goal of organs-on-chips is to clone functional units of a certain organ rather than complete organs, in order to obtain realistic but simple *in-vitro* models. With the possibility to replicate the human organ's physiology and potentially the human body as a whole, the organ-on-a-chip model can become a primary model for the study of toxicity, pharmacokinetics and pharmacodynamic of drug compounds.

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