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Organs-On-A-Chip: A New Tool for Drug Discovery

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Abstract : Novel micro fluidic tools allow new ways to manufacture and test drug delivery systems, Organ-on-a-chip systems microscale recapitulations of complex organ functions promise to improve the drug development pipeline. This review highlights the importance of integrating micro fluidic networks with 3D tissue engineered models to create organ-on-a-chip platforms, able to meet the demand of creating robust preclinical screening models. Specific examples are cited to demonstrate the use of these systems for studying the performance of drug delivery vectors and there by reduce the discrepancies between their performance at preclinical and clinical trials. We also highlight the future directions that need to be pursued by the research community for these proof- of-concept studies to achieve the goal of accelerating clinical translation of drug delivery nanoparticles.
Keywords: Organs-on-a-chip**,** Animal Testing, Micro physiological Systems Technology.

1. Introduction

One of the most challenging aspects in modern drug discovery involves identification and optimization of new drug candidates. [1, 2] Drug developers constantly strive to discover and reduce the potential side effects of lead compounds and to bring new drugs to the market in an expedited manner. Conventional in vitro platforms are useful to study and identify different signal molecules (enzymes, receptors and ligands) related to a variety of physiological processes. However, such platforms rarely mimic the complicated cell-to-cell interactions in the body and do not mimic the extracellular mechanical environment. Some of the drawbacks of the conventional in vitro cell culture methods are the static conditions with excessive amounts of nutrients, and this cannot generate the time-changing mechanical or chemical stimuli (signaling molecules) that are important for the normal cellular function. On the other hand, micro fluidics can generate not only different mechanical stimuli but also concentration gradients of certain signaling molecules (including drugs) that can be applied in an automated and time-controlled manner. Thus, micro fluidics can aid the selection of potential drug candidates and in determination of drug concentrations in a more realistic and time-efficient manner when compared with standard in vitro models. Nevertheless, the use of micro fluidics and bio micro electromechanical systems(Bio MEMS) technologies could result in the development of platforms in which the cellular micro environment scan be precisely controlled, and signals commonly found in the body (i.e., mechanical, electrical and chemical) can be applied to the cellular constructs with high precision .[3,10]Since 2D cell culture models are not always reliable alternatives for mimicking the structural complexity around the cells ,organs-on-chips can also utilize three dimensional (3D) cell culture models that better mimic tissue function and architecture.[11,12] The 3D cell culture models, unlike the two dimensional(2D) monolayer cells grown in plastic (coated or not),exert force on one another and move as they do in vivo. In

Addition, cells form more prevalent gap junctions in 3D, which are important in cell communication processes, tissue integrity and function. In terms of drug diffusion, drugs in2D culture diffuse faster than in 3D

culture, where drugs need to diffuse across multi layers of cells to their final target .Moreover, cells grown in 3D also form tight junctions that bind cells tightly and block or slow the diffusion of drugs, which is not the case in 2D culture. Thus, using 3D culture in micro fluidics rather than 2D is an important aspect that needs to be implemented in micro fluidic drug-related studies. Most of the early drug development studies use animal models to predict human pharmacokinetic responses. [13]Although, animal models are still the main source of obtaining in vivo data for predicting pharmacokinetic responses in humans, there are metabolic and physiological differences between humans and animal models that cannot always predict the outcome of new drugs. These cross-species differences could be partly avoided for certain studies by using only human cells, and within a more appropriate physicochemical environment provided by the use of micro fluidic chips. [14]

Organs-on-a-chip: a new tool for drug discovery

Organ-on-a-chip

An organ-on-a-chip (OC) is a multi-channel 3-D fluidic culture chip that simulates the activities, mechanics and physiological response of entire organs and organ systems. It constitutes the subject matter of significant biomedical engineering research, more precisely in bio-MEMS. The convergence of labs-on-chips (LOCs) and cell biology has permitted the study of human physiology in an organ-specific context, introducing a novel model of in vitro multi cellular human organisms. One day, they will perhaps abolish the need for animals in drug development and toxin testing.

Although multiple publications claim to have translated organ functions onto this interface, the movement towards this micro fluidic application is still in its infancy. Organs-on-chips will vary in design and approach between different researchers. As such, validation and optimization of these systems will likely be a long process. Organs that have been simulated by micro fluidic devices include the heart, the lung, kidney, artery, bone, cartilage, skin and more.

Nevertheless, building valid artificial organs requires not only a precise cellular manipulation, but a detailed understanding of the human body's fundamental intricate response to any event. A common concern with organs-on-chips lies in the isolation of organs during testing. "If you don't use as close to the total physiological system that you can, you're likely to run into troubles" says William Heseltine, founder of Rockville, Maryland. Micro fabrication, microelectronics and micro fluidics offer the prospect of modeling sophisticated in vitro physiological responses under accurately simulated conditions.[15]

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Lab-on-chip

A lab-on-a-chip is a device that integrates one or several laboratory functions on a single chip that deals with handling particles in hollow micro fluidic channels. It has been developed for over a decade. Advantages 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. Additionally, micro fluidic flow is entirely laminar (i.e., no turbulence). Consequently, there is virtually no mixing between neighboring streams in one hollow channel. In cellular biology convergence, this rare property in fluids has been leveraged to better study complex cell

behaviors, such as cell motility in response to chemo tactic stimuli, stem cell differentiation, axon guidance, sub cellular propagation of biochemical signaling and embryonic development.[16]

Transitioning from 3D Cell-Culture Models to Organs-on-Chips

3D cell-culture models exceed 2D culture systems by promoting higher levels of cell differentiation and tissue organization. 3D culture systems are more successful because the flexibility of the ECM gels accommodates shape changes and cell-cell connections – formerly prohibited by rigid 2D culture substrates. Nevertheless, even the best 3D culture models fail to mimic an organ's cellular properties in many aspects,[2] including tissue-to-tissue interfaces (e.g., epithelium and vascular endothelium), spatiotemporal gradients of chemicals, and the mechanically active microenvironments (e.g. arteries' vasoconstriction and vasodilator responses to temperature differentials). The application of micro fluidics in organs-on-chips enables the efficient transport and distribution of nutrients and other soluble cues throughout the viable 3D tissue constructs. Organs-on-chips are referred to as the next wave of 3D cell-culture models that mimic whole living organs' biological activities, dynamic mechanical properties and biochemical functionalities. [16]

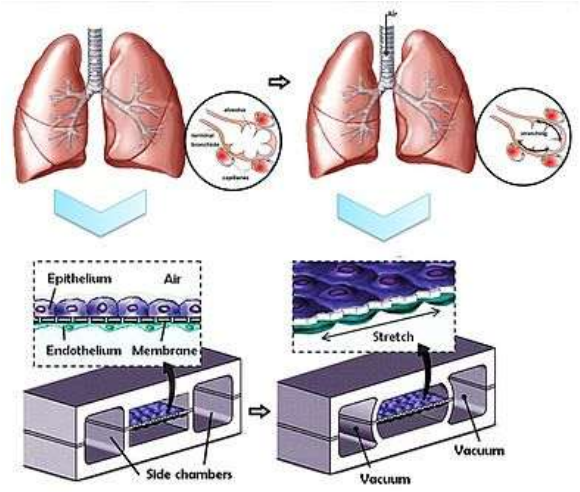
Organs

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 models.[17] Such a multifunctional micro device can reproduce key structural, functional and mechanical properties of the human alveolar-capillary interface (i.e., the fundamental functional unit of the living lung).

Example

Dongeun Huh from Wyss Institute for Biologically Inspired Engineering at Harvard describes their fabrication of a system containing two closely apposed micro channels separated by a thin (10µm) porous flexible membrane made of PDMS.[18] The device largely comprises three micro fluidic channels, and only the middle one holds the porous membrane. Culture cells were grown on either side of the membrane: human alveolar epithelial cells on one side, and human pulmonary micro vascular endothelial cells on the other.



Lung-on-a-chip – The device consists of three hollow micro channels, 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 intra pleural pressure to decrease, leading to an expansion of alveoli. This is the phenomenon essentially mimicked by this lung-on-a-chip.

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, intra pleural 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 (valued at approximately 10%), significantly increases the rate of nanoparticles translocation across the porous membrane, when compared to a static version of this device, and to a Tran swell culture system.

In order to fully validate the biological accuracy of a device, its whole-organ responses must be evaluated. In this instance, researchers inflicted injuries to the cells:

- **Pulmonary inflammation**

Pulmonary inflammatory responses entail a multistep strategy, but alongside an increased production of epithelial cells and an early response release of cytokines, the interface should undergo an increased number of leukocyte adhesion molecules.[19] In Hush's experiment, the pulmonary inflammation was simulated by introducing medium containing a potent pro inflammatory mediator. Only hours after the injury was caused, the cells in the micro fluidic device subjected to a cyclic strain reacted in accordance with the previously mentioned biological response.

- **Pulmonary infection**

Living E-coli bacteria was used to demonstrate how the system can even mimic the innate cellular response to a bacterial pulmonary infection. The bacteria were introduced onto the apical surface of the alveolar epithelium. Within hours, neutrophils were detected in the alveolar compartment, meaning they had transmigrated from the vascular micro channel where the porous membrane had phagocytized the bacteria.

Additionally, researchers believe the potential value of this lung-on-a-chip system will aid in toxicology applications. By investigating the pulmonary response to nanoparticles, researchers hope to learn more about health risks in certain environments, and correct previously oversimplified in vitro models. Because a micro fluidic 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 than a Trans well culture system. Nevertheless, published studies admit that responses of a lung-on-a-chip don't yet fully reproduce the responses of native alveolar epithelial cells.

Heart-on-a-chip

Past efforts to replicate in vivo cardiac tissue environments have proven to be challenging due to difficulties when mimicking contractility and electrophysiological responses. Such features would greatly increase the accuracy of in vitro experiments.

Micro fluidics has already contributed to in vitro experiments on cardio myocytes, which generate the electrical impulses that control the heart rate. [20]For instance, researchers have built an array of PDMS micro chambers, aligned with sensors and stimulating electrodes as a tool that will electrochemically and optically monitor the cardio myocytes' metabolism. [21]Another lab-on-a-chip similarly combined a micro fluidic network in PDMS with planar microelectrodes, this time to measure extracellular potentials from single adult urine cardiomyocytes.[22]

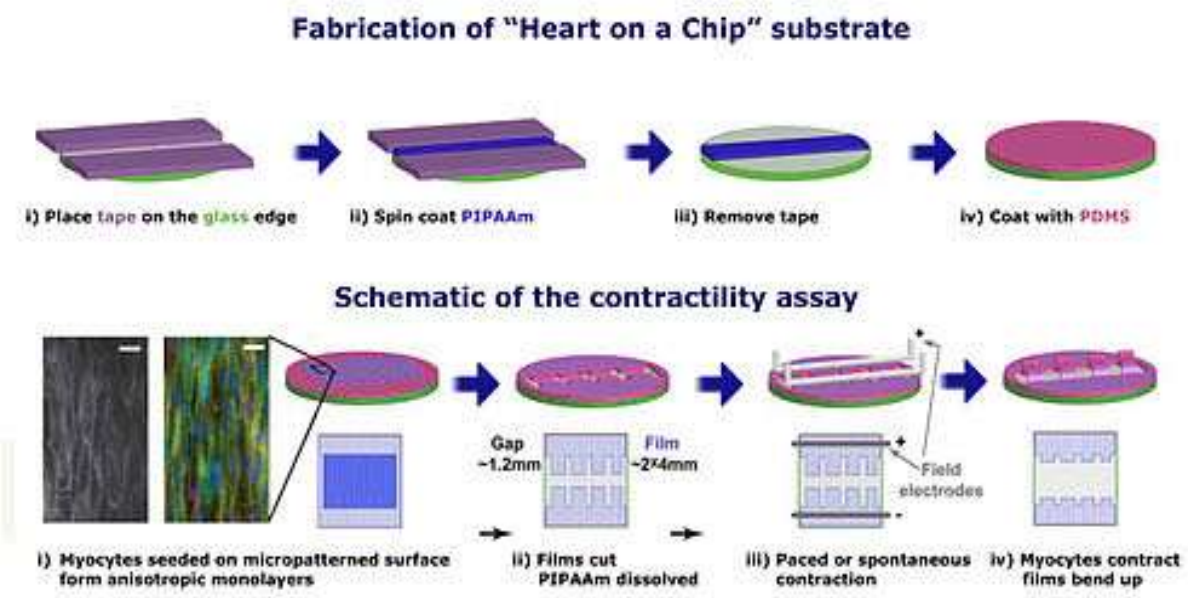


Fig .1: Heart on a chip

Preparation of the Heart-on-a-chip substrate and contractility test samples – 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 (in the realm of their respective needs).

A reported design of a heart-on-a-chip claims to have built "an efficient means of measuring structure-function relationships in constructs that replicate the hierarchical tissue architectures of laminar cardiac muscle. [23]This chip determines that the alignment of the myocytes in the contractile apparatus made of cardiac tissue and the gene expression profile (affected by shape and cell structure deformation) contributes to the force produced in cardiac contractility. This heart-on-a-chip is a bio hybrid construct: an engineered anisotropic ventricular myocardium is an elastomeric thin.

The design and fabrication process of this particular micro fluidic 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. A spin coat layer of PNIPA 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. Muscular thin films (MTF) enable cardiac muscle mono layers to be engineered on a thin flexible substrate of PDMS.[24] In order to properly seed the 2D cell culture, a micro contact printing technique was used to lay out a fibronectin "brick wall" pattern on the PDMS surface. Once the ventricular myocytes were seeded on the functionalized substrate, the fibronectin pattern oriented them to generate 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. 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 "platform for quantification of stress, electrophysiology and cellular architecture. [23]

Kidney-on-a-chip

Renal cells and nephrons have already been simulated by micro fluidic devices. "Such cell cultures can lead to new insights into cell and organ function and be used for drug screening. A kidney-on-a-chip device has the potential to accelerate research encompassing artificial replacement for lost kidney function. Nowadays, dialysis requires patients to go to a clinic up to three times per week. A more transportable and accessible form of treatment would not only increase the patient's overall health (by increasing frequency of treatment), but the whole process would become more efficient and tolerable.[25] artificial kidney research is striving to bring transportability, wear ability and perhaps implantation capability to the devices through innovative disciplines: micro fluidics, miniaturization and nanotechnology. [26]

Example – nephrons-on-a-chip

The nephrons is the functional unit of the kidney and is composed of a glomerulus and a tubular component.[27] Researchers at MIT claim to have designed a bio artificial device that replicates the function of the nephrons glomerulus, proximal convoluted tubule and loop of Henle.

Each part of the device has its unique design, generally consisting of two micro fabricated layers separated by a membrane. The only inlet to the micro fluidic device is designed for the entering blood sample. In the glomerulus' section of the nephrons, 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.[28]

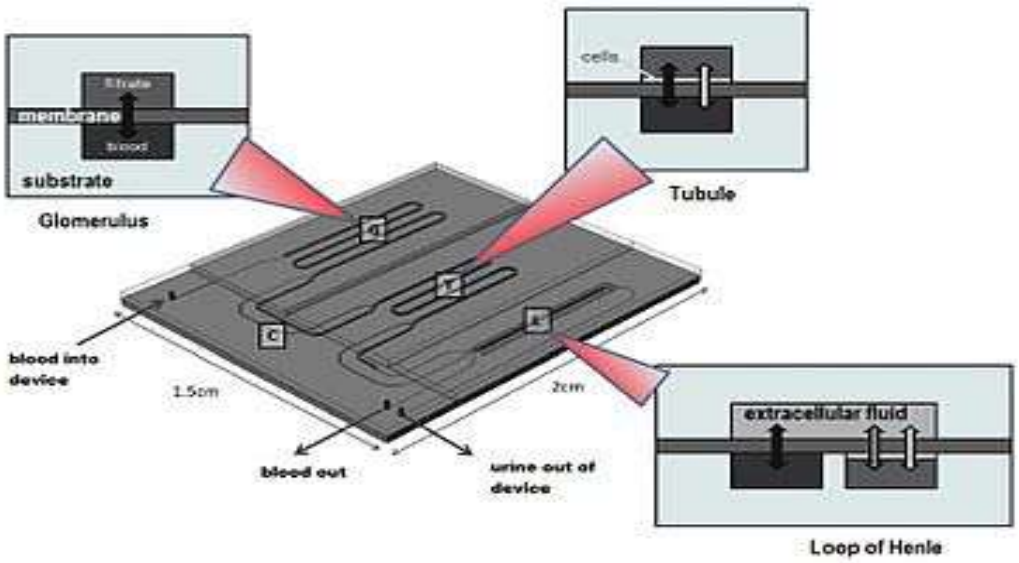


Fig .2: Nephrons-on-a-chip

Schematic of a nephrons-on-a-chip device with cross-sections of 3 functional units – C –Connector; G – Glomerulus; T – Tubule; L – Henle's loop Black arrows: passive transport White arrows : cell-mediated active transport

In the tubules, some substances are added to the filtrate as part of the urine formation, and some substances reabsorbed out of the filtrate and back into the blood. The first segment of these tubules is the proximal convoluted tubule. This is where the almost complete absorption of nutritionally important substances takes place. In the device, this section is merely 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 there absorption 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 cells and medullary collecting duct cells.[27]

One step towards validating the micro fluidic device's simulation of the full filtration and re absorption behavior of physiological nephrons 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 re absorption 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.[29]

One recent report illustrates biomimic nephrons on hydro gel micro fluidic devices with establishing the function of passive diffusion. [30]The complex physiological function of nephrons is achieved on the basis of interactions between vessels and tubules (both are hollow channels). [31]However, conventional laboratory techniques usually focus on 2D structures, such as Petri-dish that lacks capability to recapitulate real physiology that occurs in 3D. Therefore, the authors developed a new method to fabricate functional, cell-lining and perusable micro channels inside 3D hydro gel. The vessel endothelial and renal epithelial cells are cultured inside hydro gel micro channel and form cellular coverage to mimic vessels and tubules, respectively. They employed co focal microscope to examine the passive diffusion of one small organic molecule (usually drugs) between the vessels and tubules in hydro gel. The study demonstrates the beneficial potential to mimic renal physiology for regenerative medicine and drug screening.

Artery-on-a-chip

Cardiovascular diseases are often caused by changes in structure and function of small blood vessels. For instance, self-reported rates of hypertension suggest that the rate is increasing, says a 2003 report from the National Health and Nutrition Examination Survey. [32]A micro fluidic platform simulating the biological response of an artery could not only enable organ-based screens to occur more frequently throughout a drug development trial, but also yield a comprehensive understanding of the underlying mechanisms behind pathologic changes in small arteries and develop better treatment strategies. Axel Gunther from the University of Toronto argues that such MEMS-based devices could potentially help in the assessment of a patient's micro vascular status in a clinical setting (personalized medicine).[33]

Conventional methods used to examine intrinsic properties of isolated resistance vessels (arterioles and small arteries with diameters varying between 30 µm and 300 µm) include the pressure myography technique. However, such methods currently require manually skilled personnel and are not scalable. An artery-on-a-chip could overcome several of these limitations by accommodating an artery onto a platform which would be scalable, inexpensive and possibly automated in its manufacturing.

Example

An organ-based micro fluidic platform has been developed as a lab-on-a-chip onto which a fragile blood vessel can be fixed, allowing for determinants of resistance artery malfunctions to be studied.

The artery microenvironment is characterized by surrounding temperature, transmural pressure, and luminal & abluminal drug concentrations. The multiple inputs from a microenvironment cause a wide range of mechanical or chemical stimuli on the smooth muscle cells (SMCs) and endothelial cells (ECs) that line the vessel's outer and luminal walls, respectively. Endothelial cells are responsible for releasing vasoconstriction and vasodilator factors, thus modifying tone. Vascular tone is defined as the degree of constriction inside a blood vessel relative to its maximum diameter.[34] Pathogenic concepts currently believe that subtle changes to this microenvironment have pronounced effects on arterial tone and can severely alter peripheral vascular resistance. The engineers behind this design believe that a specific strength lies in its ability to control and simulate heterogeneous spatiotemporal influences found within the microenvironment,[33] whereas myography protocols have, by virtue of their design, only established homogeneous microenvironments. They proved that by delivering phenylephrine through only one of the two channels providing super fusion to the outer walls, the drug-facing side constricted much more than the drug opposing side.

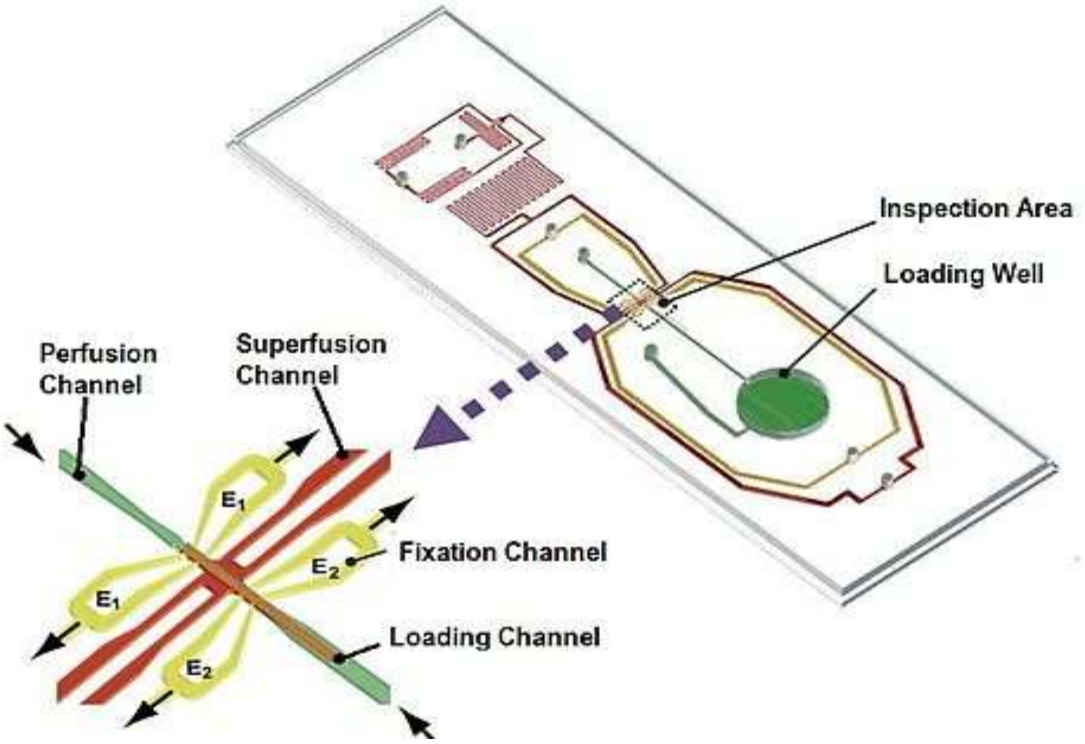


Fig .3: Artery-on-a-chip

Artery-on-a-chip and detail of inspection area – The green micro channel is used for loading the artery segment, and perfusion (delivery of nutrients to the luminal walls); the fixation channels in yellow are used to adjust the positioning of the organ in the inspection zone by applying sub-atmospheric pressures at each end; in red is the super fusion channel, used to deliver nutrients to the abluminal wall of the artery.

The artery-on-a-chip is designed for reversible implantation of the sample. The device contains a micro channel network, an artery loading area and a separate artery inspection area. There is a micro channel used for loading the artery segment, and when the loading well is sealed, it is also used as a perfusion channel; to replicate the process of nutritive delivery of arterial blood to a capillary bed in the biological tissue.[35] another pair of micro channels serves to fix the two ends of the arterial segment. Finally, the last pair of micro channels is used to provide super fusion flow rates, in order to maintain the physiological and metabolic activity of the organ by delivering a constant sustaining medium over the abluminal wall. A thermoelectric heater and a thermo resistor are connected to the chip and maintain physiological temperatures at the artery inspection area.

The protocol of loading and securing the tissue sample into the inspection zone helps understand how this approach acknowledges whole organ functions. After immersing the tissue segment into the loading well, the loading process is driven by a syringe withdrawing a constant flow rate of buffer solution at the far end of the loading channel. This causes the transport of the artery towards its dedicated position. This is done with closed fixation and super fusion in/outlet lines. After stopping the pump, sub-atmospheric pressure is applied through one of the fixation channels. Then after sealing the loading well shut, the second fixation channel is subjected to a sub-atmospheric pressure. Now the artery is symmetrically established in the inspection area, and a transmural pressure is felt by the segment. The remaining channels are opened and constant perfusion and super fusion are adjusted using separate syringe pumps.[33]

Human-On-A-Chip

Researchers are working towards building a multi-channel 3D micro fluidic cell culture system that compartmentalizes microenvironments in which 3D cellular aggregates are cultured to mimic multiple organs in the body.[36] Most organ-on-a-chip models today only culture one cell type, so even though they may be valid models for studying whole organ functions, the systemic effect of a drug on the human body is not verified.

Conceptual schematic of a human-on-a-chip – Designing a whole body biomimetic device will potentially correct one of the most significant limitations on organs-on-chips: the isolation of organs.

In particular, an integrated cell culture analog (μCCA) was developed and included lung cells, drug-metabolizing liver and fat cells. The cells were linked in a 2D fluidic network with culture medium circulating as a blood surrogate, thus efficiently providing a nutritional delivery transport system, while simultaneously removing wastes from the cells[37] "The development of the μCCA laid the foundation for a realistic in vitro pharmacokinetic model and provided an integrated biomimetic system for culturing multiple cell types with high fidelity to in vivo situations", claim C. Zhang et al. They have developed a micro fluidic human-on-a-chip, culturing four different cell types to mimic four human organs: liver, lung, kidney and fat. [38]They focused on developing a standard serum-free culture media that would be valuable to all cell types included in the device. Optimized standard media are generally targeted to one specific cell-type, whereas a human-on-a-chip will evidently require a common medium (CM). In fact, they claim to have identified a cell culture CM that, when used to perfuse all cell cultures in the micro fluidic device, maintains the cells' functional levels. Heightening the sensitivity of the in vitro cultured cells ensures the validity of the device, or that any drug injected into the micro channels will stimulate an identical physiological and metabolic reaction from the sample cells as whole organs in humans.

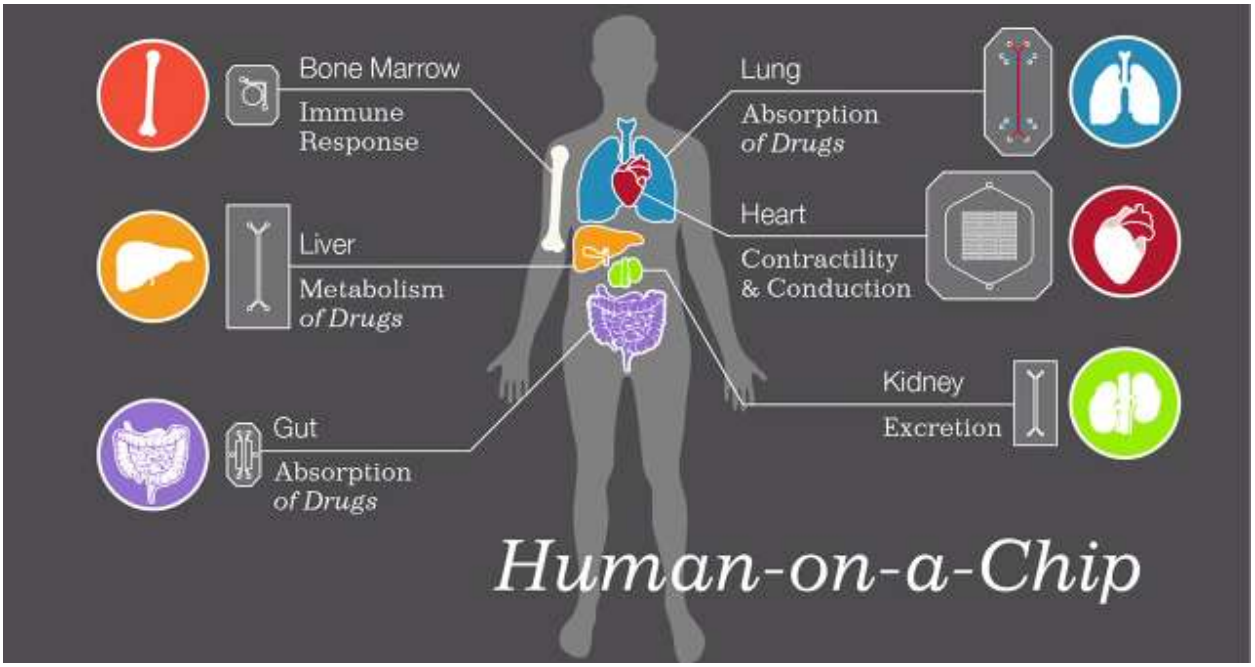


Fig .4: Human-On-A-Chip

With more extensive development of this kind of chip, pharmaceutical companies will potentially be able to measure direct effects of one organ's reaction on another. For instance, the delivery of biochemical substances would be screened to confirm that even though it may benefit one cell type, it does not compromise the functions of others. It is probably already possible to print these organs with 3D printers, [38] but the cost is too high. Designing whole body biomimetic devices addresses a major reservation that pharmaceutical companies have towards organs-on-chips, namely the isolation of organs. As these devices become more and more accessible, the complexity of the design increases exponentially. Systems will soon have to simultaneously provide mechanical perturbation and fluid flow through a circulatory system. "Anything that requires dynamic control rather than just static control is a challenge", says Takayama from the University of Michigan.[39]

Replacing animal testing with organs-on-chips

In the early phase of drug development, animal models were the only way of obtaining in vivo data that would predict the human pharmacokinetic responses. However, experiments on animals are lengthy, expensive and controversial. For example, animal models are often subjected to mechanical or chemical techniques that

simulate human injuries. There are also concerns with regards to the validity of such animal models, due to deficiency in cross-species extrapolation. [40]Moreover, animal models offer very limited control of individual variables and it can be cumbersome to harvest specific information.

Therefore, mimicking a human's physiological responses in an in vitro model needs to be made more affordable, and needs to offer cellular level control in biological experiments: biomimetic micro fluidic systems could replace animal testing. The development of MEMS-based biochips that reproduce complex organ-level pathological responses could revolutionize many fields, including toxicology and the developmental process of pharmaceuticals and cosmetics that rely on animal testing and clinical trials.[41]

Organs-on-a-Chip and Micro physiological Systems

Advances in tissue engineering and artificial organ systems are expected to have a major impact on drug discovery, screening, and assessment of efficacy and safety, and to facilitate basic research of cellular and sub cellular mechanisms.

Coupled systems of *in vitro* micro fabricated organs-on-a-chip and human organ constructs (HOCs) containing small populations of human cells are being de-eloped to address the formidable pharmacological and physiological gaps between monolayer cell cultures, animal models, and humans. These gaps present challenges not only in tissue and micro fluidic engineering, but also in systems biology: how does one model, test, and learn about the communication and control of biological systems at the scale of individual organs on chips? Algometric scaling provides some guidance, but appropriate biochemical and functional scaling of multiple organs and a universal cell-culture medium are critical to proper systems function and valid pharmacological interpretation.

The ultimate potential of engineered organs or micro physiological systems will be realized by more complex, powerful, and integrated systems capable of recapitulating inter- and intra-organ signaling and dynamics. The operation of a single HOC requires a perfusion system functionally integrated into a tubing circuit with connections for medium inflow and outflow, pressure measurement, sample removal; pumps to recalculate and replenish culture medium; and sensors, actuators, and control electronics to regulate flow rates, pH, temperature, and gas mixtures. Real-time measurements of glucose, lactate, pH and oxygen allow the quantification of HOC metabolism, including aerobic vs. anaerobic processes, receptor activation, and other activities that affect metabolism.

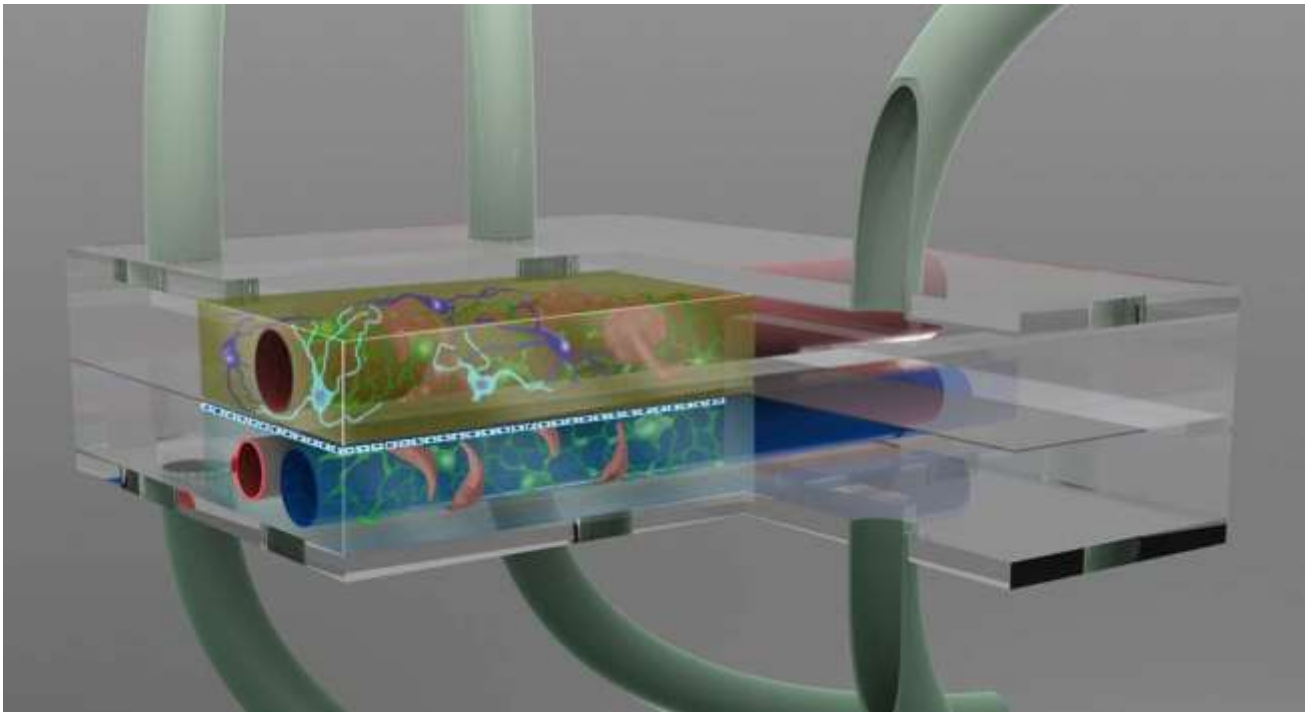


Fig .5: Planar BBB NVU Chip

Concept of Planar BBB NVU Chip

The Vanderbilt Institute for Integrative Bio systems Research and Education (VIIBRE) is currently building a planar Neurovascular Unit (NVU) on a Chip under NIH funding. VIIBRE is also building a cardiac papillary muscle-on-a-chip utilizing DTRA funding and additional supplementary funding was received to research a Retinal-Blood-Barrier-on-a-Chip.

In addition, VIIBRE is developing a hardware and multi-organ-chip integration platform for the DTRA X.C.E.L. program in collaboration with organ-on-chip teams from Charité – Universitätsmedizin Berlin (liver), Harvard University (heart), Los Alamos National Laboratory (lung, organ integration and integrated validation), University of California San Francisco (kidney). [42]

"Organs-on-Chips" technology for drug testing

- Collaboration
- Hot Topics

Fig.6: "Organs-on-Chips" technology for drug testing

Animal studies to evaluate the efficacy and safety of novel drugs are something we're committed to reducing, wherever possible. As Vice President Drug Safety and Metabolism at AstraZeneca, I am interested by new opportunities to use predictive science technologies to find innovative ways to ensure patients safety. An example of this is our recent collaboration with the Wyss Institute at Harvard University, which has allowed us to integrate their "Organs-on-Chip" technology into drug development. Organs-on-Chip are self-contained units about the size of a memory stick that contain hollow micro fluidic channels lined by living human cells. The human cells recreate the physiological functions of organs without using animal models.

More than ten Organs-on-Chip are currently under development, including a lung, liver, kidney, gut, skin, blood-brain barrier, and bone marrow-on-a-Chip. There is also a major effort to integrate these organ chips into "a virtual human body on-chips" that mimics whole body physiology. This will provide more predictive and useful measures of the efficacy and safety of potential new drugs in humans so that we can better understand how a medicine might ultimately impact patients.

There are other significant advantages to using this technology such as reductions in both the cost and the timelines of bringing a drug to market. Along with the cost benefits, the major downstream effects of the use of these Organs-on-Chips are improve.[43]

"Organs-on-Chips" technology for drug

There are other significant advantages to using this technology such as reductions in both the cost and the timelines of bringing a drug to market. Along with the cost benefits, the major downstream effects of the use of these Organs-on-Chips are improvements in patient safety and reductions in the need for animal testing.

Professor Don Ingber, Founding Director of the Wyss Institute for Biologically Inspired Engineering, is really excited about initiating studies that will validate the technology. Professor Ingber has recently been awarded the prestigious 3Rs Prize from the UK's National Centre for the Replacement, Refinement and Reduction of Animals in Research for the development of the Lung-on-Chip.

The work underlying the concept of Organ on a Chip and this collaboration is very much in line with our commitment to the 3Rs of animal research. We see the use of the Organs-on-Chips as providing an innovative avenue to replace animal models in early drug testing.

This relationship marks another exciting collaboration between an academic centre of excellence and AstraZeneca. Organs-on-Chips are at the very forefront of bioengineering and I am personally excited by this collaboration. The use of Organs-on-Chips is an innovative, ground-breaking approach that will hopefully foster closer links between the Bioengineering experts at the Wyss Institute and AstraZeneca, whilst providing beneficial reductions in the need for animal testing. In addition to AstraZeneca's exploration of Organs-on-Chip systems, we are also investing in computational approaches and also state-of-the art screening systems based on human stem cells. Together these new technologies are enhancing our efficiency and effectiveness in making translational safety predictions and reducing our requirement for in vivo studies. Ments in patient safety and reductions in the need for animal testing.

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Limitations

It is current culture platforms used for developing drug delivery systems Several parameters need to be studied for developing nanoparticles for clinical use. These include studying the fate of the nanoparticles inside the body and its toxicological effects, the mode of binding and internalization at the cellular level, the stability of the nanoparticles with respect to various physical and chemical conditions of the body, and, most importantly, the efficacy when compared to free drugs Large-batch synthesis, toxicity assessment and efficacy screening are the major levels at which clinical translation of nano therapeutics faces set-back. On the manufacturing front, scaling the small lab synthesis techniques to the large-scale production of nanoparticles has been challenging for the pharmaceutical companies .Meanwhile, screening for the toxicity and efficacy suffers from the paucity of preclinical models that would robustly predict the nanoparticles' behavior inside the human body .For simultaneous evaluation of the above-mentioned parameters, predictive in vitro platforms are

essential while developing drug delivery vectors. The current gold standard for preclinical testing of nano therapeutics is in vivo studies. These do not accurately predict human responses due to inter-species difference in genetic makeup, along with being extremely time-consuming, expensive, low-throughput and raising ethical concerns. The resolution for whole-animal imaging methods is limited, hindering visualization during transport of the theranosticagents in the target tissue. Being unable to reproduce its preclinical performance, many drug delivery systems which pass the preclinical phase fail to address the toxicity and efficacy effects when compared to their free drug counterparts in human clinical trials. Strikingly, the main reason cited for this effect is the use of animal models for optimization during drug carrier design .which brings back the obvious drawback of a certain degree of physiological irrelevance between human and animal models. Animal models need to be complemented with sophisticated in vitro plat forms to fill this gap. In current in vitro studies, drug delivery carriers are commonly tested in two-dimensional (2D) monolayer cell culture models. These 2D cultures involve growing on top of a flat substrate (e.g., glass or polystyrene) a monolayer of single or multiple cell types that are either freshly isolated from human/animal tissues (primary cells) or are already established, immortalized cell lines. In these setups, drug delivery systems are usually mixed with culture media and directly applied on the cell monolayer's, after which cellular responses are recorded. Among several published studies, the work of Xia and colleagues on the cellular uptake of gold nanoparticles (AuNPs) by SK-BR-3 breast tumor cells stands out by devising novel testing method. After culturing the cells on a piece of glass.[45]

Future Direction & Conclusion

Pharmaceutical companies involved in drug discovery continuously aim to both reduce the cost of their research and speed up the development of new drugs. In this process, pre-clinical animal studies are expensive and time-consuming, while not assuring human in vivo significance. In vitro organ-on-a-chip platforms can revolutionize this process by making it less expensive, while representing a reliable way for drug discovery. However, further advances in the field are needed to overcome engineering and biological challenges before entirely assessing their value as an efficient and robust platform for screening Nano therapeutics. The final goal is to develop a body-on-a-chip platform for systemic evaluation of drug delivery vectors. But current efforts are directed toward the development of individual organ platforms limiting their.[46]

Range of applications to a specific organ's functions. For example, liver being the most relevant organ for drug toxicity and metabolism, the development of a functional liver-on-a-chip system is the most important challenge currently faced by researchers. As mentioned in, several attempts have been developed in this direction, but the available platforms are yet unable to fully capture the complexities of in vivo drug metabolism in a robust manner. For developing a universal platform, the flexibility and modularity of this technology, based on extremely versatile micro fabrication techniques, should be exploited. The possibility of culturing different cell types employing the identical standard setups could lead to the development of several organ models on demand. The results obtained with such systems could be easily compared, and relevant drug-specific cell responses can be quickly noticed. In this context, the simplicity of the setup and the implementation of several parallel experiments will be a key aspect (e.g., for testing drug–dose responses as well as different target organs). [47]

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