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ORGAN ON A CHIP: A NEW TOOL FOR DRUG DISCOVERY

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ABSTRACT
Microfluidics is a science and technology that precisely manipulates and processes microscale fluids. It is commonly used to precisely control microfluidic (10−9 to 10−18L) fluids using channels that range in size from tens to hundreds of microns and is known as a "lab-on-a-chip". The microchannel is small, but has a large surface area andhigh mass transfer, favoring its use in microfluidic technology applications including low regent usage, controllable volumes, fast mixing speeds, rapid responses, and precision control of physical and chemical properties. Microfluidics integrate sample preparation, reactions, separation, detection, and basic operating units such as cell culture, sorting and cell lysis . For these reasons, interest in OOAC (Organ-On-A-Chip)has intensified. OOAC combines a range of chemical, biological and material science disciplines and was selected as one of the "Top Ten Emerging Technologies" in the World Economic Forum.

KEYWORDS: Organ On A Chip, Lung On A Chip, Brain On A Chip, Heart On A Chip, Kidney On A Chip, Prostate On A Chip, Pancreas On A Chip, microfluidics.

INTRODUCTION
An organ-on-a-chip is a micro-scale system used for mimicking the human body environment. The goal for organ-on-a-chip is to develop human tissue models for disease modeling and drug testing. They use microfluidics, along with cells, to imitate the physiological and mechanical conditions experienced in the body.

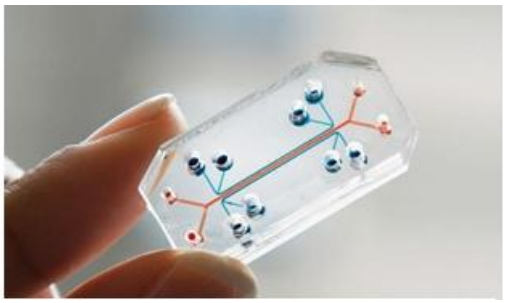


Figure 1: Organ on A Chip Design.

It is a multi-channel 3-D microfluidic cell culture, integrated circuit (chip) that simulates the activities, mechanics and physiological response of an entire organ or an organ system, a type of artificial organ. 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 multicellular human organisms. One day, they will perhaps abolish the need for animals in drug development and toxin testing.

Requirements for Such Futuristic Technology
Human physiology is the science of studying the functions of the human body and its organ systems. This is of great significance to our understanding of the dysfunction and pathogenesis of the body, and therefore closely aligns with the fields of medicine, drug development and toxicology. The most relevant and direct methods for studying human physiology are in vivo experiments that study human or model organisms. Bodily functions rely on the interaction and adaptation of many lower-level components such as tissues, cells, proteins and genes. It is therefore challenging to reveal the underlying mechanisms of physiological phenomena simply through in vivo studies. In addition, drug development and toxicology require the assessment of the physiological effects of thousands of compounds. Due to the limitations of low-throughput in vivo testing, biologists use in vitro cell culture. Cell culture refers to the growth and maintenance of cells in a controlled environment. For decades, traditional two-dimensional (2D) cell culture systems formed an important platform for life science research. Using 2D systems, the functions of various cells are studied by culturing cells or cell products. However, 2D systems fail to accurately

simulate the physiological manifestations of living tissues/organs, intra-organ interactions and micro environmental factors and often require verification in vivo animal models. Due to species differences, animal experiments often fail to replicate human experiments, and due to both high costs and ethical issues, the use of animals as models for drug testing has come under scrutiny. In preclinical testing, an inadequate description of the human tissue environment may lead to inaccurate predictions of the combined effects of overall tissue function. OOAC was designed to overcome these shortcomings by providing more physiological model systems. OOAC was proposed as a future replacement technology for experimental animalmodels.

Organ-on-a-chip technology provides a novel in vitro platform with a possibility of reproducing physiological functions of in vivo tissue, more accurately than conventional cell-based model systems. Many newly arising diseases result from complex interaction between multiple organs. By realizing different organ functions on a chip, organ-on-a- chip technology is a potentially useful for building models of such complex diseases.

Organ-On-A Chip Design & Concept

❖ **Design Concept**

Culture systems require the control of external and internal cell environments. OOAC combined with micromachining and cell biology can control external parameters and accurately simulate physiological environments. Dynamic mechanical stress, fluid shear and concentration gradients are required on the chip. Cell patterning should also be realized to fully reflect physiological processes.

❖ **Fluid shear force**

Microfluidics enables the dynamic culture of cells through micro-pump perfusion, which facilitates the administration of nutrients and timely waste discharge. The dynamic environment in which cells are located is more comparable to in vivo conditions than static culture. In addition, fluid shear stress induces organ polarity. Importantly, OOAC exerts necessary physical pressure on the normal biological functions of endothelial cellsby activating cell surface molecules and associated signaling cascades. Similarly, the incorporation of fluid into the OOAC device permits biological assessments at the single organ level. The OOAC system summarizes flow through a simple "rocker" on a chip fluid motion, or through a more complex programmable "pulsatile" format, arranged in a single loop for organization-specific configurations.

❖ **Concentration gradient**

At the micro scale level, the fluid acts primarily as a laminar flow, resulting in a stable gradient of biochemical molecules, controlled both spatially and temporally. Various biochemical signals driven by concentration gradients exist in biological phenomena, including angiogenesis, invasion, and migration. Microfluidics simulate complex physiological processes in the human body by altering flow velocity and channel geometryusing micro valves and micro-pumps to achieve stable, three-dimensional (3D) biochemical concentration gradients.

❖ **Dynamic mechanical stress**

Normal day-to-day organ pressure includes blood pressure, lung pressure, and bone pressure. These pressures play a major role in maintaining mechanically stressed tissues such as skeletal muscle, bone, cartilage and blood vessels. Microfluidics enable the use ofelastic porous membranes to create periodic mechanical stresses. This mechanical stimulation is considered a key determinant of differentiation during physiological processes.

❖ **Cell patterning**

The organization of the human body requires a complex and ordered arrangement of multiple cells to form a functional whole body interactions. Microfluidics control cell patterning for the construction of in vitro physiological models with complex geometries. Surface modifications, templates, and 3D printing contribute to cell patterning on the chip. The 3D printing method enables multi-scale cell patterning by permitting the formation of hydrogel scaffolds with complex channels. The advantage of 3D printing is to allow user-defined digital masks to provide versatility in cell patterns, critical for the in vitro reconstruction of the cellular microenvironment. Li et al. developed methods to achieve rapid heterotypic cell patterning on glass chips using controlled topological manipulations. This method combines a polyvinyl acetate coating, carbon dioxide laser ablation, and continuous cell seeding techniques on a glass chip. This method enables controlled epithelial–mesenchymal interactions. In addition, mesenchymal cells with similar properties can also be patterned on glass chips. This method can be helpful for large-scale investigation and pharmaceutical testing of cutaneous epithelial– mesenchymal interaction and can also be applied to the patterning of other cells.

Emerging Technologies In Organ-On-A-Chips

The OOAC involves four key components, including

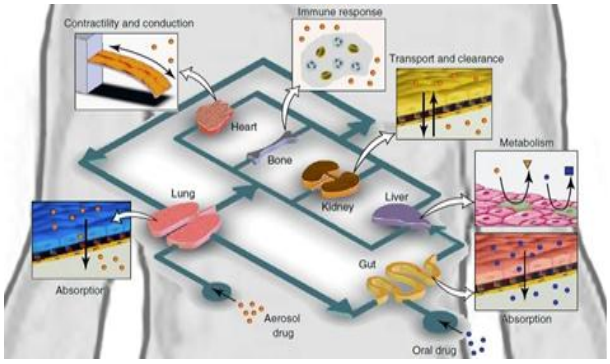
(1) Microfluidics; - The microfluidic component refers to the use of microfluidics to deliver target cells to a pre-designated location and includes a system of culture fluid input and waste liquid discharge during the culture process. Typically, this component is characterized by miniaturization, integration, and automation

(2) Living cell tissues;- The living cell tissue component refers to components that spatially align a particular cell type in the case of 2D or 3D systems. The 3D arrangements are typically created by the addition of biocompatible materials such as hydrogels. These materials can prevent mechanical damage and shape three- dimensional arrangements

(3) Stimulation or drug delivery

(4) Sensing -The sensing component for detecting and

compiling data can be an embedded sensing output component or a transparent chip based visual function evaluation system. A meaningful human-on-chip cell model cannot be described and accessed without microsensors-mediated reading of the metabolic state at characteristicpoints in the system.



Materials Needed For Organ-In-A-Chip and Outside Devices

According to Abu-Dawas, S., Alawami, H., Zourob, M., and Ramadan, Q. et. al., the organ-on-a-chip needs to be manufactured using a material which does not influencethe cellular microenvironment components and maintain a stable fluid connection. The most popular material used is known as polydimethylsiloxane (PDMS). This material is a polymetric and synthetic elastomer based on carbon and silicon. The manufacturing itself is made of mixing the liquid PDMS with an agent which helps with the solidification of PDMS. The mixture is then poured into a mold giving the form of the chip. After the mixture hardens the chips, body can be either glued to lass or to another chip. The PDMS became popular due to many of its properties, being transparent it helps the user since they can see how the OOC behaves. The material is cheap and is known to have a reduced cytotoxicity, making it easy to use in this application. Because PDMS doesn't degrade it issometimes replaced by other materials. However there have been researchers who tried to 3D print the chip, requiring only one step, and avoiding any troublesome events that may occur using multiple step methods that would require the solubilization of the initialscaffold.

Due to the different structure of each organ, different biomaterials are required in order to obtain good results. For example, collagen is widely used because of its advantages, but it requires some mechanical support without which the collagen remains intact for a shorttime. In some cases, external equipment is required in order to obtain the best results. Firstly, it is necessary to control the external flow of the micro and nanofluids. For this pressure generators and different types of pumps are used. The best way to control flow is to use hydrostatic pressure. Pressure generators are usually simple devices, having incorporated a pressure source, for example a compressor, a pressure regulator and a manometer to measure the current pressure value. Even though the system is simple it does have major setbacks mostly contained in its limited response time. There are, however, methods to bypass this problem, using a pressure multiplexer the pressure can be changed much faster. Another upgrade to pressure generators can be done by adding flux sensors which change the control signal from pressure to flow. The viscosity and density of the cell culture changes after a certain number of days. When constructing the chip this property has to be remembered since studies show that the pressure and stress of the walls increased significantly in the analyzed models.

Pressure syringes are another system usually used for flow control. They are usually used in perfusions, but scientists have also adopted them in microfluidic research. They have the advantage of being able to control the flow without being affected by perturbations caused by fluid resistance. Same as the pressure generator, the settling time is high because of the small values the flow pulses use.

Commonly, there are two types of pumps used for flow control, the first one being simplepumps used for liquids which have the disadvantage of having a nonlinear model.However, there exists a linear equation, used as an alternative in order to model the system easier. The systems usually require a good sensor in order to detect small flow fluctuations. The other type of pumps are the electro-osmotic ones, which do not have flow fluctuation problems and are resistant to high counter pressure, but have the disadvantage of requiring low conductivity liquids in order to work properly.

Strengths and Limitations of Organ-On-A-Chip

Strengths

- The first major strong point of the organ-on-a-chip concept is the accelerated research it can generate. Since the cost of manufacturing the chips is rather cheap, it is possible to fabricate using in-house accessories without any specialized equipment, many drugs and doses of drugs can be tested at the same time. This may prove helpful when a new drug is discovered, not needing test subjects and at the same time not meeting ethnic concerns.

- Another strong suit of the OoAC concept is the close resemblance of the tissue microenvironment it replicates. When comparing the OoAC with simple Petri recipient microsystems, the OoAC comes out on top due to the 3D structure which is an important element of the test's reliability.

- Additionally, the microfluidic chips are user friendly and, in some cases, can be portable are capable to assess many physiological questions. Due to their small size multiple microfluidic systems can be integrated on one chip, saving space and money at the same time.

Limitations

- The first disadvantage considered is the presence of

the surface effect. Since the dimensions of the fluids are very small, the surface effects dominate the volume effect. This may reflect in poor quality of the analysis and some of the product of interest may be adsorbed. Since laminar flow is present at the intersection of multiple fluids the relevant fluids might not mix properly.

- Another limitation of these platforms is represented by the fact that, in some experiments, there is a need for special instruments in order to obtain reliable results.

Analyzed Organs

Organs are made up of different types of cells corresponding to their role in the human body. Since different organs have different roles the structure of their cells is changes depending on cell types. Thus, the OoAC must be constructed to best suit the microenvironment the cells experimented on-

Liver on A Chip

The liver is one of the most important organs in the human body, mostly for its several functions to maintain normal physiological activities. In order to cope with the damages which may be caused to it through chemical or physical means, it has great regenerative capacity. In some cases, the injuries may be too severe, mostly caused by adverse reactions from different drugs or diseases. Before the OoAC technology there were not many great *invitro* models to be used. Most of the drugs were tested *in vivo* on animal subjects. The subjects are exposed to the adverse reactions of the drugs and, in some cases, being fatal to them. The OoAC technology detects if the different drugs harm liver cells. The methodology is to seed the chip with liver cells and apply the drug in different channels. The chip is observed and if the cells die the drug is deemed to harmful to be used *in vivo*. Because of this, it can be considered that this system can be useful for hepatotoxicity studies.

There are several liver-on-chip methods used:

- Liver Sinusoid which replicates the lacuna between adjacent liver plates;
- Liver Lobule, considered the smallest functional unit of the liver;
- Zonation in the lobule is an optimized segregation of the liver functions in spatial and temporarily defined zones.

Some scholars like Esch, M. B., Ueno, H. et. al., think that the engineering behind the liver-on-chips requires to integrate scaffolding materials for achieving 3D cultures, for cell growth and to maintain the interactions between the cells. Those components may be natural or synthetic, but are practiced in many applications. Such applications may vary from drug testing to behavior analysis. On a microfluidic chip it was observed that coating the chip with collagen supports the hepatocyte growth and adhesion. For a layer-by-layer deposition the cells were coated with nanofilms of fibronectin and gelatin which resulted in the reconstruction of liver tissue with high cellular function. Others found that by seeding hepatocytes, endothelial cells and stellate cells on fiber membranes the tissues formed secreted albumin and urea for several days.

Most liver-on-a-chip models that study nano-toxicological effects are 2D systems. One of the more predominant roles of the liver is to filter the blood and remove the toxins from it. Due to the nature of this task the toxins can accumulate in the liver causing changes to its function and cellular morphology. One of the improvements to the liver-on-a-chip concept include the use of perfusions, the effect being the prolonged lifespan of the cells and improved drug metabolism. An innovative idea is the use of multi-species liver-on-a-chip system which uses the liver cells from multiple animals, this is done to prove that each species can react differently to certain drugs. In the example given, the drug killed the human liver cells, but the rat cells remained alive.

Liver-on-a-chip is a 3D *in vitro* hepatic micro physiological system aiming to recreate the conditions of liver tissue on a microscopic scale. An ideal liver-on-a-chip is a higher- throughput system capable of mimicking conditions of hepatocytes and the dynamic physicochemical hepatic environment.

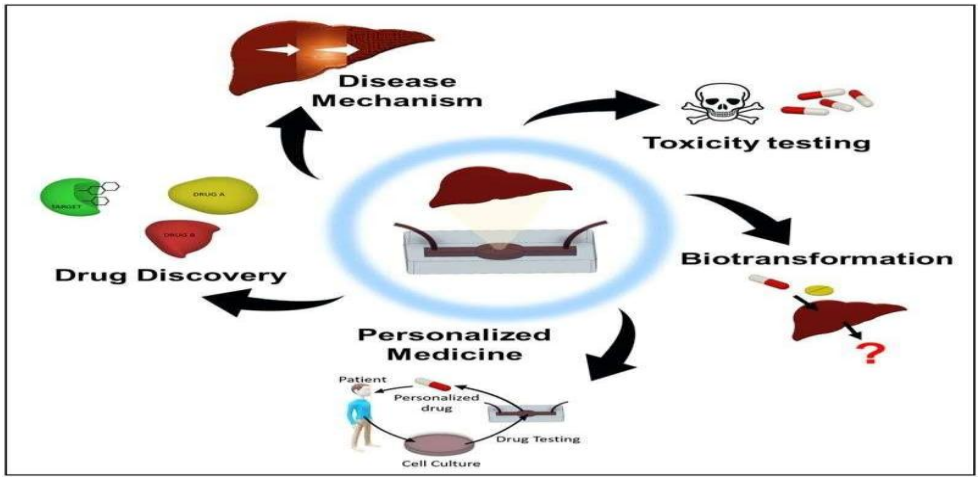


Figure 3: Liver on a Chip.

Lungs on A Chip

Lungs do not possess great regenerative properties, as such any damage caused to them might be permanent. In addition, pulmonary cancer is one of the more common types of cancers that may occur to a human, smokers being especially affected by this disease. Also, many areas have a high degree of air pollution, such as china, causing heavy damage to the lungs. Under these conditions the research on lung cells is deemed important. Theincreasing popularity of OoAC means that researchers already try to model the lungs on this technology. There are certain researchers who accomplished this task using microfluidic devices and harvesting lung cells on these devices. They simulate the behavior of the lungs by increasing and decreasing the pressure of the device, while the cells replicate the behavior of the lungs. The researchers Lu, R. X. Z., and Radisic, M. et. al. observed that the cells would react as if they were in their normal environment in the body, the alveoli contracted and expanded mimicking the breathing, while the porous membrane expanded and contracted according to the pressure given. Having this technology, different reactions can be analyzed, ranging from the behavior of tumor cellsto the reaction of lung cells to smoking or other environmental toxic agents. Different drugs can be tested as well to determine their effects on some diseases and to observe their potential side effects. OoAC can be used to study the effects of different environmental factors on human organs. Such a study is done by Rick and Milica who analyze the adverse effects of nanoparticles and their toxicity. The nanoparticles are the remains from plastic and other non-biodegradable materials with the addition of fossil fuels and nitric oxide. It is proven that these have a toxic effect by the experiments done. Lungs exposed to these toxins are more likely to develop pulmonary complications like pulmonary fibrosis, asthma or pulmonaryedema. Some of the problems occur due to the rapid advancements in technology and people not understanding how toxic can some of the newly developed fuels can be, shouldbe considered for application on environmental toxicology. Most experiments regarding OoAC are focused on either toxicity or diseases around the alveoli, only a fraction of the entire respiratory system, however there are different airway-on-a-chip models and even innovative approaches which use 3D printing and constructing in addition to the OoAC concept.

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. In Huh'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 microfluidic device subjected to a cyclic strain reacted in accordance with the previously mentioned biological response.

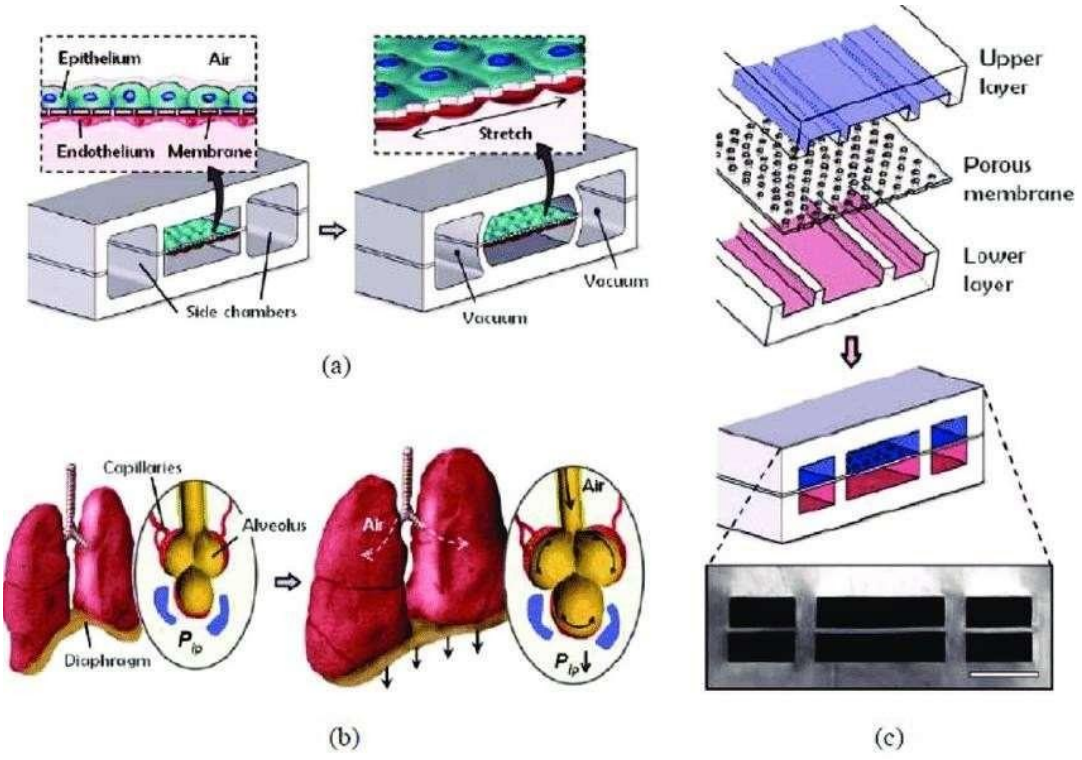
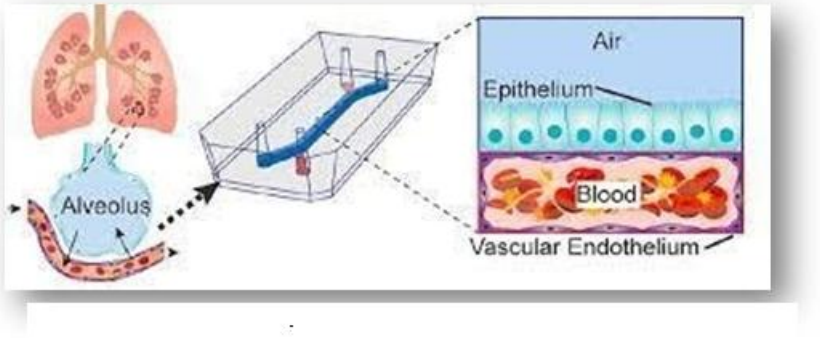


Figure 4: Mechansim Of Lung-On-A Chip.

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.



Kidney on A Chip

The kidney is responsible for the maintenance of osmotic pressure drug excretion. Kidney toxicity leads to an irreversible loss of renal filtration highlighting the need for drug screening systems. Filtration and reabsorption take place in the nephrons that consist of the glomerulus, renal capsule, and renal tubule. Microfluidics can simulate the fluid environment that support tubular cell growth, and provides porous membrane support for the maintenance of cell polarity.

Nephron on A Chip

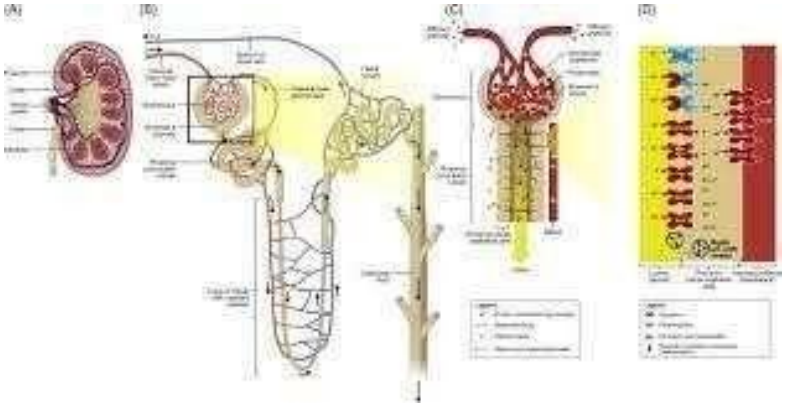


Figure 6: Nephron On A Chip.

The nephron is the functional unit of the kidney and is composed of a glomerulus and a tubular component. 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 microfluidic device is designed for the entering blood sample. In the glomerulus' section of the nephron, 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, 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 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.

One step towards validating the microfluidic device's simulation of the full filtration and reabsorption behavior of a physiological nephron 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 200–400 mM.

One recent report of Ramadan, Q., and Gijs, M. A. M et. al illustrates a biomimic nephron on hydrogel microfluidic devices with establishing the function of passive diffusion.The complex physiological function of nephron is achieved on the basis of interactions between vessels and tubules (both are hollow channels). 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 perfusable microchannels inside 3D hydrogel. The vessel endothelial and renal epithelial cells are cultured inside hydrogel microchannel and form cellular coverage to mimic vessels and tubules, respectively. They employed confocal microscope to examine the passive diffusion of one small organic molecule (usually drugs) between the vessels and tubules in hydrogel. The study demonstrates the beneficial potential to mimic renal physiology for regenerative medicine and drug screening.

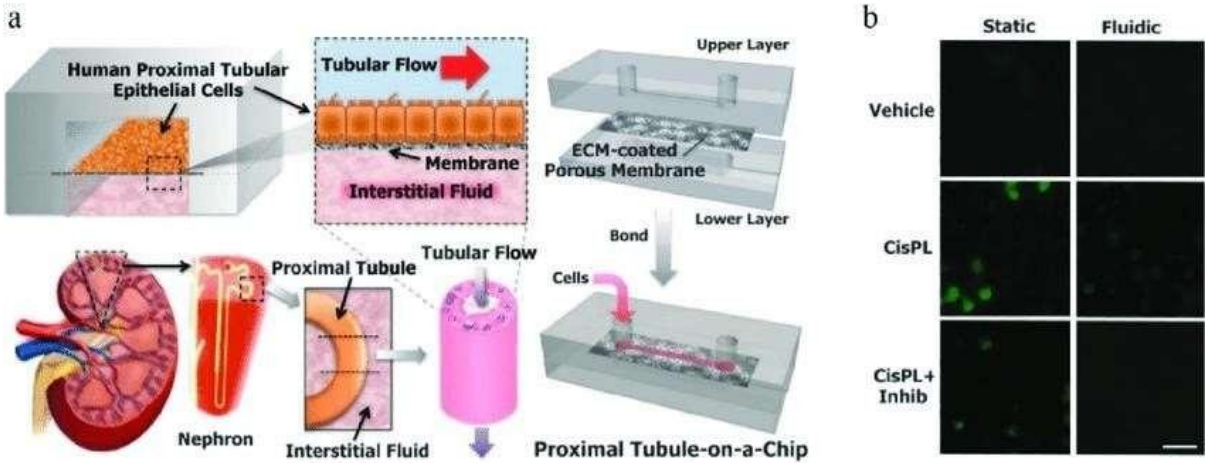
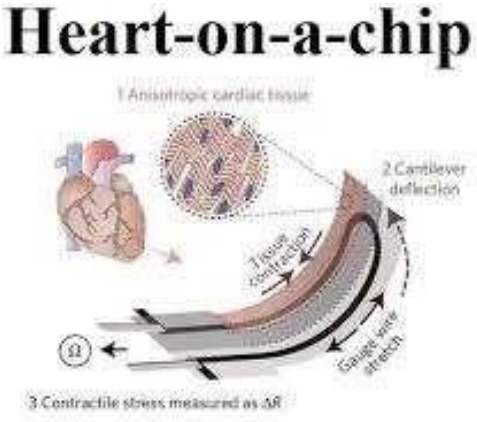


Figure 7: Kidney On A Chip.

Heart on A Chip

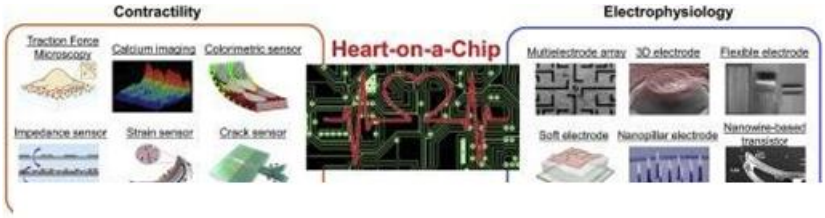
Cardiovascular deaths are the leading cause of human mortality. The emergence of microfluidics has enabled in vitro bionic studies of cardiac tissue. The myocardium is a major component of the heart. The beating of cardiomyocytes (CMs) can be used to directly assess drug effects and is directly related to heart pumping. Microfluidics has already contributed to in vitro experiments on cardiomyocytes, which generate the electrical impulses that control the heart rate. For instance, researchers have built an array of PDMS microchambers, aligned with sensors and stimulating electrodes as a tool that will electrochemically and optically monitor the cardiomyocytes' metabolism. Another lab-on-a-chip similarly combined a microfluidic network in PDMS with planar microelectrodes, this time to measure extracellular potentials from single adult murine cardiomyocytes.

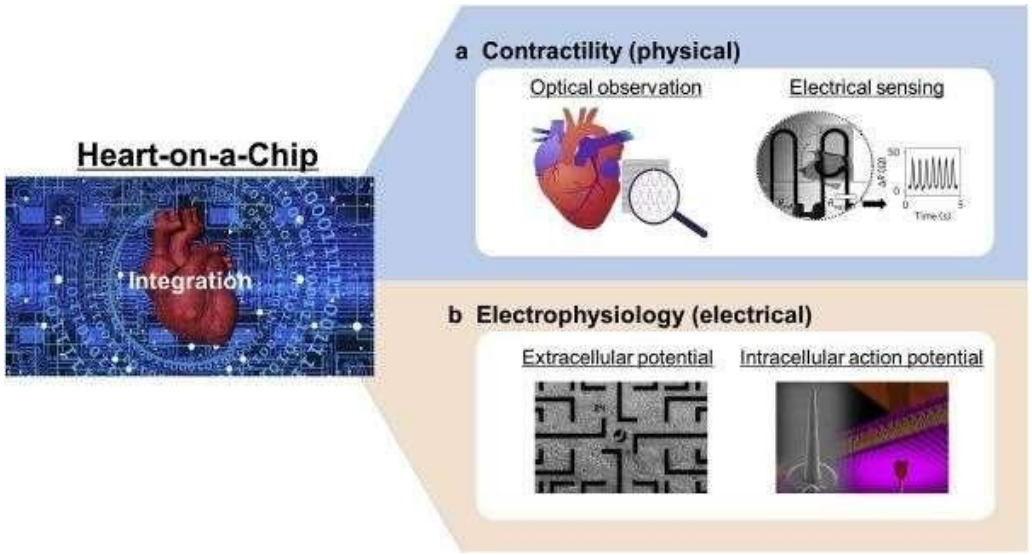


A reported design of a heart-on-a-chip claims t0 have built "an efficient means of measuringstructure- function relationships in constructs thatreplicate the hierarchical tissue architectures of laminar cardiac muscle." This chip determines that the alignment of the myocytes in the contractileapparatus 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 biohybrid construct: an engineeredanisotropic ventricular

myocardium is anelastomeric thin film.

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 contourthe 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 monolayers to be engineered on a thin flexible substrate of PDMS. In order to properly seed the 2D cell culture, a microcontact 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 generatean anisotropicmonolayer.





BRAIN ON A CHIP

The brain might be the most complex organ and the hardest to create an OoC after. The functionality of the brain is far too complex and differs from person to person, as such in most papers the models do not cover this aspect, but rather go over and analyze cell ratios use the transporting properties of the brain and analyze the neuro vascular unit and the blood brain barrier. What makes the cell ratios of neurons and glial cells is that the ratio changes depending on the brain section which is analyzed.

There have been successful *in vitro* cell cultures by Bang, S., Jeong, S., Choi, N., and Kim, H. N. et. al. in open environment such as glass substrates or Petri dishes. The technique used in the culture of the BoC is called multistep flithography. It consists of separating the soma and axon. The technique allowed the study of the axon, its regeneration and treatment using different drugs on the axon. The models created are used to study neurodegenerative diseases and understanding the behavior andthe effectsit has on the brain cells.

Three categories of BoC development are developed:

- 3D high content systems used to mimic the brain tissue environment;
- Interconnected multichip system which is used to simulate interactions between multiple cells and organs;
- High-throughput systems, screening of experimental conditions.

Microfluidic devices have been paired with organotypic slices to improve culture viability.The standard procedure for culturing organotypic brain slices (around 300 microns in thickness) uses semi-porous membranes to create an air-medium interface, but this technique results in diffusion limitations of nutrients and dissolved gases. Because microfluidic systems introduce laminar flow of these necessary nutrients and gases, transport is improved and higher tissue viability can be achieved. In addition to keeping standard slices viable, brain-on-a-chip platforms have allowed the successful culturing of thicker brain slices (approximately 700 microns), despite a significant transport barrier due to thickness. As thicker slices retain more native tissue architecture, this allows

brain- on-a-chip devices to achieve more "*in vivo*-like" characteristics without sacrificing cell viability. Microfluidic devices support high-throughput screening and toxicological assessments in both 2D and slice cultures, leading to the development of novel therapeutics targeted for the brain.One device was able to screen the drugs pitavastatin and irinotecan combinatorically in glioblastoma multiform (the most common form of human brain cancer).These screening approaches have been combined with the modeling of the blood-brain barrier (BBB), a significant hurdle for drugs to overcome when treating the brain, allowing for drug efficacy across this barrier to be studied *in vitro*. Microfluidic probes have been used to deliver dyes with high regional precision, making way for localized microperfusion in drug applications.Because microfluidic devices can be designed with optical accessibility, this also allows for the visualization of morphology and processes in specific regions or individual cells. Brain- on-a-chip systems can model organ-level physiology in neurological diseases, such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis more accurately than with traditional 2D and 3D cell culture techniques. The ability to model these diseases ina way that is indicative of *in vivo* conditions is essential for the translation of therapies and treatments. Additionally, brain-on-a-chip devices have been used for medical diagnostics, such as in biomarker detection for cancer in brain tissue slices.

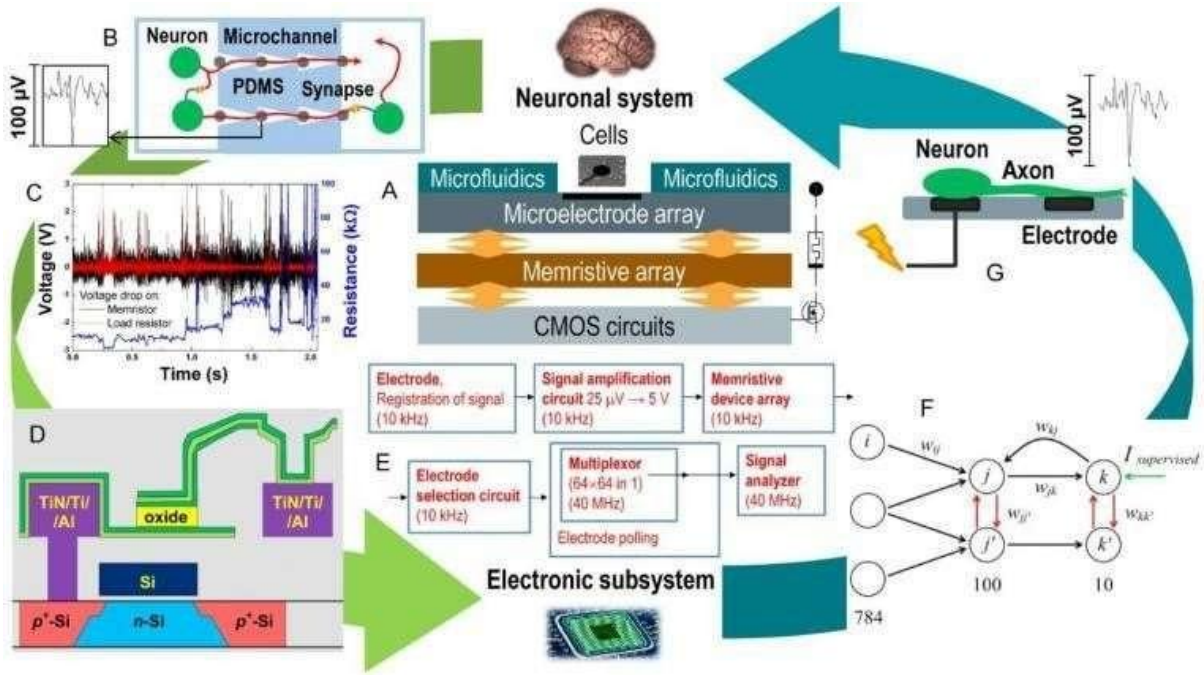


Figure 10: Brain On a Chip Technology.

PROSTATE ON A CHIP

Recreation of the prostate epithelium is motivated by evidence suggesting it to be the site of nucleation in cancer metastasis. These systems essentially serve as the next step in thedevelopment of cells cultured from mice to two and subsequently three-dimensional human cell culturing. PDMS developments have enabled the creation of microfluidic systems that offer the benefit of adjustable topography, gas and liquid exchange, as well as an ease of observation via conventional microscopy.

Researchers at the University of Grenoble Alpes have outlined a methodology that utilizes such a microfluidic system in the attempt to construct a viable Prostate epithelium model.The approach focuses on a cylindrical microchannel configuration, mimicking the morphology of a human secretory duct, within which the epithelium is located. Various microchannel diameters were assessed for successful promotion of cell cultures, and it was observed that diameters of 150-400 µm were the most successful. Furthermore, cellular adhesion endured throughout this experimentation, despite the introduction of physical stress through variations in microfluidic currents.

The objective of these constructions is to facilitate the collection of prostatic fluid, along with gauging cellular reactions to microenvironmental changes. Additionally, prostate- on-a-chip enables the recreation of metastasis scenarios, which allows the assessment of drug candidates and other therapeutic approaches. Scalability of this method is also attractive to researchers, as the reusable mold approach ensures a low-cost of production.

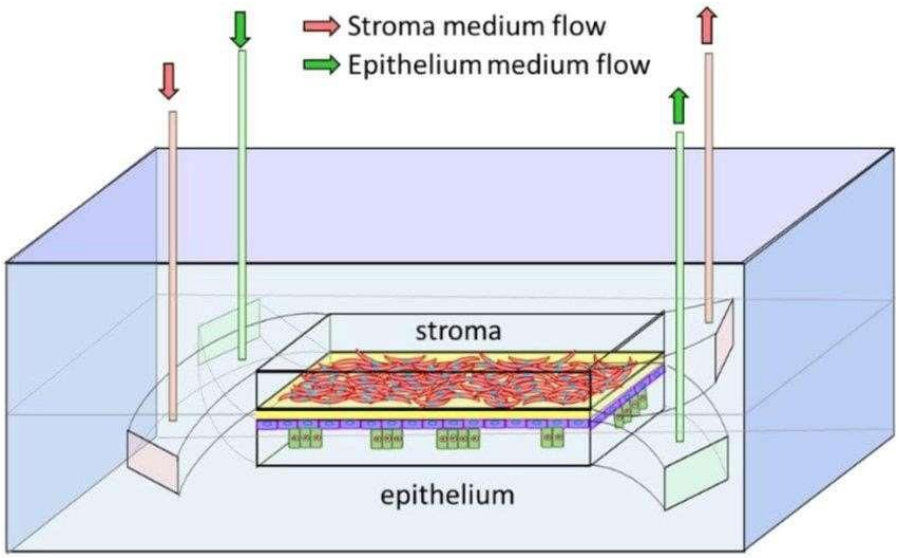


Figure 11: PROSTATE ON A CHIP.

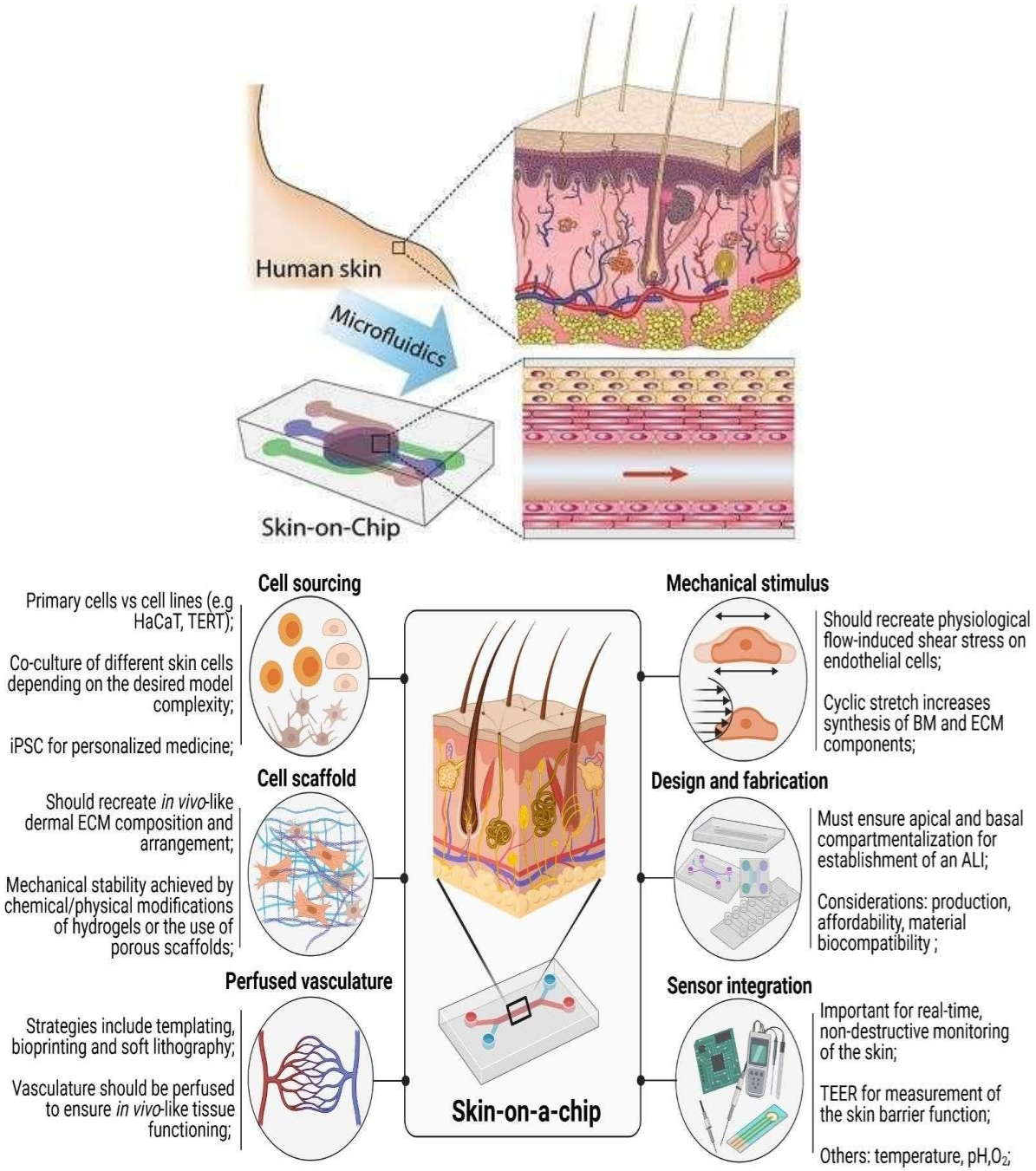
SKIN ON A CHIP

Human skin is the first line of defense against many pathogens and can itself be subject to a variety of diseases and issues, such as cancers and inflammation. As such, skin-on-a- chip (SoC) applications include testing of topical pharmaceuticals and cosmetics, studying the pathology of skin diseases and inflammation, and "creating noninvasive automated cellular assays" to test for the presence of antigens or antibodies that could denote the presence of a pathogen. Despite the wide variety of potential applications, relatively little research has gone into developing a skin-on-a-chip compared to many other organ-on-a-chips, such as lungs and kidneys. Issues such as detachment of the collagen scaffolding from microchannels, incomplete cellular differentiation, and predominant use of poly (dimethysiloxane) (PDMS) for device fabrication, which has been shown to leach chemicals into biological samples and cannot be mass- produced stymie standardization of a platform. One additional difficulty is the variability of cell-culture scaffolding, or the base substance in which to culture cells, that is used in skin-on-chip devices. In the human body, this substance is known as the extracellular matrix.

The extracellular matrix (ECM) is composed primarily of collagen, and various collagen- based scaffolding has been tested in SoC models. Collagen tends to detach from the microfluidic backbone during culturing due to the contraction of fibroblasts. One study attempted to address this problem by comparing the qualities of collagen scaffolding from three different animal sources: pig skin, rat tail, and duck feet. Other studies also faced detachment issues due to contraction, which can problematic considering that the process of full skin differentiation can take up to several weeks. Contraction issues have been avoided by replacing collagen scaffolding with a fibrin-based dermal matrix, which did not contract. Greater differentiation and formation of cell layers was also reported in microfluidic culture when compared to traditional static culture, agreeing with earlier findings of improved cell-cell and cell- matrix interactions due to dynamic perfusion, or increased permeation through interstitial spaces due to the pressure from continuous media flow. This improved differentiation and growth is thought to be in part a product of shear stress created by the pressure gradient along a microchannel due to fluid flow, which may also improve nutrient supply to cells not directly adjacent to the medium. In static cultures, used in traditional skin equivalents, cells receive nutrients in the medium only through diffusion, whereas dynamic perfusion can improve nutrient flow through interstitial spaces, or gaps between cells.This perfusion has also been demonstrated to improve tight junction formation of the *stratum corneum*, the tough outer layer of the epidermis, which is the main barrier to penetration of the surface layer of the skin.

Dynamic perfusion may also improve cell viability, demonstrated by placing a commercialskin equivalent in a microfluidic platform that extended the expected lifespan by several weeks. This early study also demonstrated the importance of hair follicles in skin equivalent models. Hair follicles are the primary route into the subcutaneous layer for topical creams and other substances applied to the surface of the skin, a feature that morerecent studies have often not accounted for.

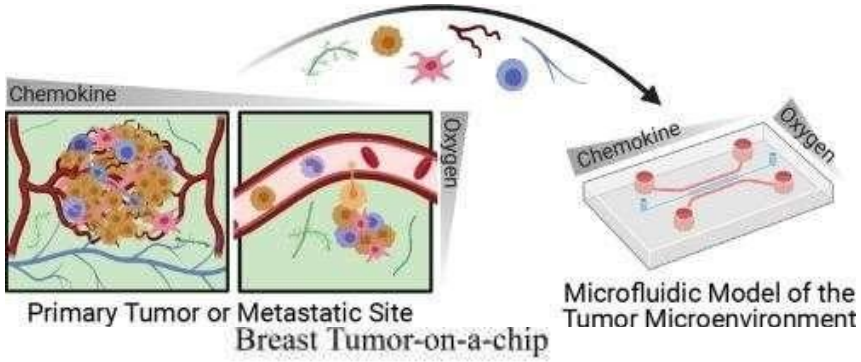
One study developed of Jusoh, N., Ko, J., and Jeon, N. L. et. al, a SoC consisting of three layers, the epidermis, dermis, and endothelial layer, separated by porous membranes, to study edema, swelling due to extracellular fluid accumulation, a common response to infection or injury and an essential step for cellular repair. It was demonstrated that pre-application of Dex, a steroidal cream with anti- inflammatory properties, reduced this swelling in the SoC.

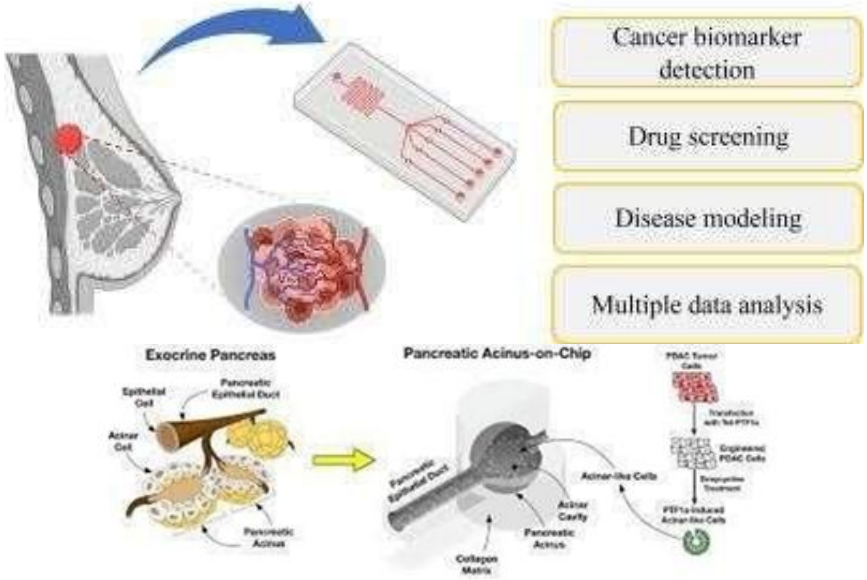


Breast Tissue and Tumor on a Chip Concept

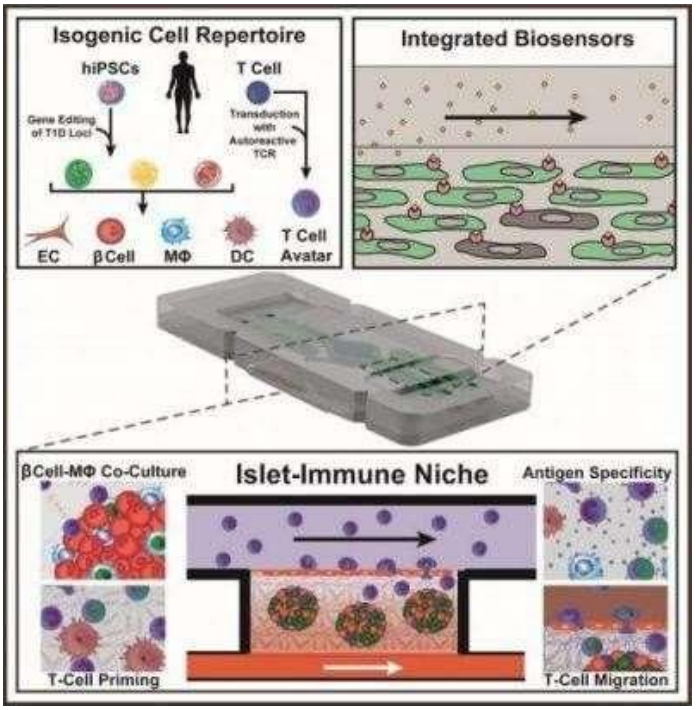
Understanding breast cancer anatomy is useful to better study breast cancer, by Chen, Y., Gao, D., Wang, Y., Lin, S., and Jiang, Y. et. al a frequent malignancy among women. Its danger comes from its invasive properties and the difficulty in curing it. Different 2D models have been used throughout the years with little success, due to the models not being able to replicate in an accurate way the manner in which the tumor behaves. As in many other fields different *in vivo* experiments were studied on animals with the downside of these models being the lack of reproducibility ofthe obtained results.

The concept of tumor-on-chip (ToC) is a microfluidic 3D system designed to mimic the tumor behavior, biological activities, mechanical properties and different responses of thetumor cells. In the case of breast cancer these ToC devices are used for drug screening bystudying different chemotherapeutic drugs and its effects on the model, or for personalized medicine when using primary cells. Knowing the factors that can induce resistance to tumor cells can lead to the discovery of new therapeutic strategies for treating this disease. With this knowledge, the risks of the treatments can be reduced and personalized drugs can be the next step of curing malignancies. For example, these models can be used to study the interaction of the malignant clone with adipocytes considering that this type of cells are prone for tumorigenesis and can promote drug response.





Pancreas on a Chip



Pancreatic cancer is one of the typesof cancer having one of the worse prognostics, due to its high resistance to the drugs used on it and the difficulty of removing the tumor through surgery. The poor outcomes of this disease are

believed to be caused by the cancer cells which have high invasiveness features, thus the cancer reaching advanced stages in a short period oftime.

Researchers Wikswo, J. P., Curtis, E. L., Eagleton, Z. E., Evans, B. C., Kole, A., Hofmeister, L. H. et. al, took the OoAC approach by using clear, flexible plastic chips which contained microfluidic channels. In order to replicate the behavior of the disease they use pancreatic cancer cells harvested from mice in one channel and seed the neighboring channel with human endothelial cells. The chip is then observed in order to study the behavior of these cells. After the experiment is concluded the same procedure is executed, but instead of mouse pancreatic cancer cells, human cells are used. The same behavior is seen suggesting that across different species the behavior for the disease does not change. This discovery means that whatever treatment is used on test subjects, such as mice, would work on humans as well. OoAC areused in the search for a cure as well, by adding experimental cures to the cells of the chipthen observing their behavior. In some cases, the spread of the cancer cells is slowed indicating that that substance should be tested *in vivo*.

Diabetes is a common disease that has a compromising effect on the functioning of the pancreas. There are several ways of better understanding this disease, one of the methodsis with the aid of the OoAC. The researchers use OoAC technology to study the behavior of insulin producing cells in order to better understand the phenomenon. It is expected thatwith help of OoAC the efforts of studying and understanding the disease should be accelerated.

Microsystem Types

Hydrogel Based Microsystems

Liu, H., Wang, Y., Cui, K., Guo, Y., Zhang, X., and Qin, J.In their natural cells are surrounded by a network called Extracellular Matrix (ECM). The function and structure of the cells are given by the surrounding molecules. In cell culture this environment is missing, this resultsin different ways to mimic the environment. One of the more popular approaches uses the hydrogel. Hydrogels are made of different types of polymers which are swollen with water. Cells can be added inside the hydrogel by mixing the cell solution with the hydrogel before it gets solid. The cells are encapsulated in the gelwhich will be their ECM.

Significant advances in materials,

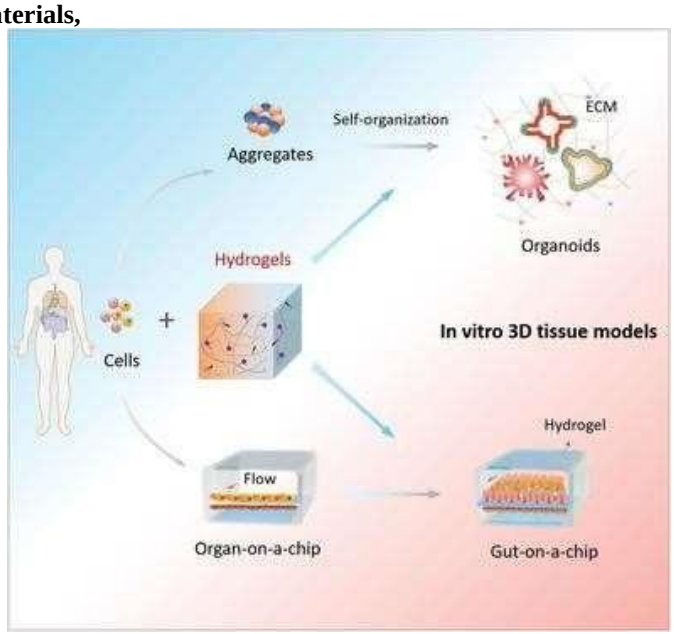


Figure 12: Hydrogel & Organ in a Chip.

microscale technology, and stem cell biology have enabled the construction of 3D tissues and organs, which will ultimately lead to more effective diagnostics and therapy. Organoids and organs-on-a-chip (OOAC), evolved from developmental biology and bioengineering principles, have emerged as major technological breakthrough and distinct model systems to revolutionize biomedical research and drug discovery by recapitulating the key structural and functional complexity of human organs in vitro. There is growing interest in the development of functional biomaterials, especially hydrogels, for utilization in these promising systems to build more physiologically relevant 3D tissues with defined properties. The remarkable properties of defined hydrogels as proper extracellular matrix that can instruct cellular behaviors are presented. The recent trend where functional hydrogels are integrated into organoids and OOAC systems for the construction of 3D tissue models is highlighted. Future opportunities and perspectives in the development of advanced hydrogels toward accelerating organoids and OOAC research in biomedical applications are also discussed.

- **Biometric Microfluidic Devices**

Microfluidic devices are used in research centers, clinics

and hospitals being powerful tools for monitoring, diagnostics and drug delivery. They were integrated in the OoC technology and revolutionized the fields of drug screening and toxicology studies.

The concept itself is associated with the control and manipulation of liquids at a small scale, as small as microliters. The advantages of microfluidics are vast: small sample volume, scalability, predictable fluid dynamics, high resolution and sensitivity, low cost, short analysis time and a large application pool. The applications vary from drug testing, to pollutant detection, going as far as repelling biowarfare adversities. In the OoC technology alone, microfluidics has a large number of useful applications: study of different organ and tissue activity, cell culture, drug delivery, wound healing, diagnostics.

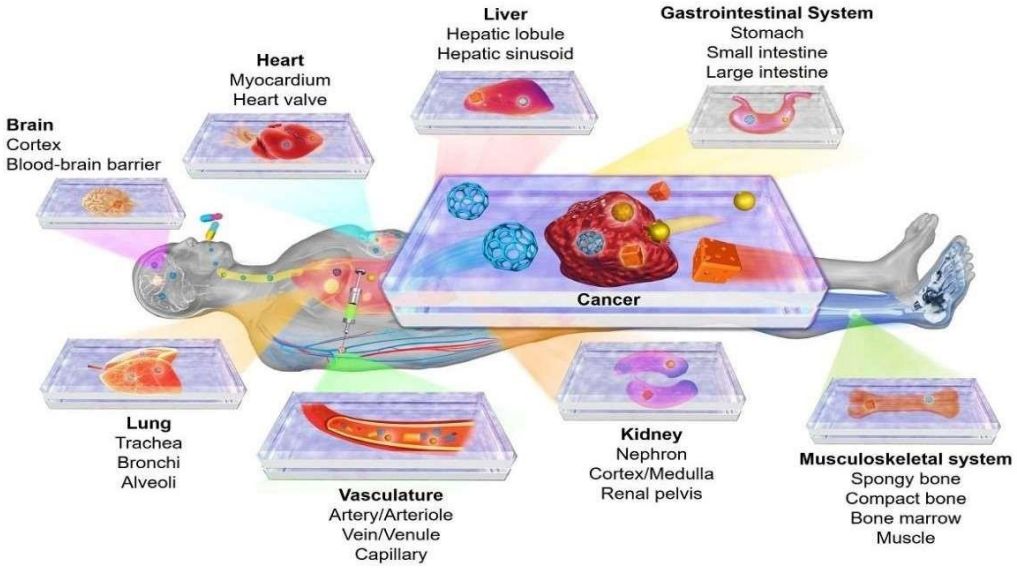
Microfluidic devices are used for numerous different organs and systems of organs such as the respiratory system, excretory system, nervous system and many others. These systems consist of multiple organs, the devices containing different tissues and cells from each organ. For each system the role of the microfluidic devices may differ or they mighthave multiple roles

- **Personalized Organ in A Chip**

Since the OoAC research has known such a radical growth over the years, different moreprecise applications are being initiated. With the use of this concept an effort is being done in order to create personalized versions of the chip. This meaning that more specific and accurate diagnosis and drugs testing results can be obtained. The overall idea is to use as much information as possible when using the OoAC, information specific to the individual the tissue sample is harvested. For example, in the experiments, the results will also be affected by genetic, physiological and biometric parameters specific to an individual. The obtained results give a clearer, more accurate model, or simulation, of how a disease, drug or specific external factors may react with the cells in the OoAC, permitting the application of the concept of personalized medicine in clinical practice, selecting the optimal treatment scenarios for patient benefit.

Firstly, the precision treatment should be discussed. There are several factors which maymake some drugs not eligible to use, ranging from pregnancy, age, diverse medical conditions and even being a smoker or not. There have been researchers that use OoAC to compare the behavior of a healthy and of a smoker's lungs. Being able to give highly precise medication without risking the wellbeing of the patient may even save lives. There have been several reports and news of people receiving wrong medication because of the side effects the drugs they were given affected them differently due to the doctors not checking for a specific condition.

In order to test a drug on a control group, specific parameters are required and some conditional environments might be needed. If some patients need to be started on antibiotics for a bacterial infection, for each person several OoAC will be used to test the effects of different antibiotics to check which one should be used with the least side effects. The patients might react differently to the medication they receive, with this method noneof them risk to be mistreated.



CONCLUSION

The concept of OoAC has been around for a long time and they have led to a lot of new discoveries in the medical field. The applications range from disease studying to drug testing considering the complex environmental interaction among different cell types. In the present times single-organ-on-a-chip models have already been created for almost all organs, with some studies on multiple OoAC devices interconnected. One of the next development steps is the integration of sensors into the chips, which makes monitoring key physiological parameters easier. It is an important field

which with further development can lead to new and revolutionary discoveries. The human-body-on-a-chip can be considered one of them. In most cases the medicine used to cure a certain type of disease may help in the recovery of a certain organ, but there are side effects which may occur in other organs. Having a model of the human body which consists of a system of OoAC connected could put an end to animal testing and would mean a speed-up in the pharmaceutical industry. New biomaterials and techniques which may allow some of the harder and more complex physiological functions to be modeled with ease. For the fabrication of the OoAC 3D printing, soft lithography, hot embossing and injection moldingare all commonly used and each has some advantage over the other. Recently the conceptof bioprinting has been used by many researchers which could lead to more cost-efficientfabrication of the OoAC. Soft-lithography and photolithography, cost, time and design improvements are all more advantageous if bioprinting is used More importantly the different paths that are opened can reach to the automation of medical procedures and the elimination of mistreatment.

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