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ORGAN ON A CHIP - A REVIEW AND ITS ROLE IN RESEARCH

[1] M. Deepika, [2]SK. Sushma Taj, [3]K. Jahnavi, [4]B. Jeshma Kalyani, [5]K. Navaneetha

[1,3,4,5]Research Students, [2]Assistant Professor,

Department of Pharmaceutics,

Joginpally B.R. Pharmacy College, JNTUH, Bhaskar Nagar, Yenkapally, Moinabad, Telangana, India

Abstract: Organ-on-a-chip (OOAC) is a physiological organ biomimetic system constructed on a microfluidic chip and it is listed among the top 10 emerging technologies. The microenvironment of the chip mimics the organ in terms of tissue interactions and mechanical stimulation by combining cell biology, engineering and biomaterial technologies. This reflects the composition and functionality of human tissue and forecasts how the body will react to a wide range of stimuli, such as environmental factors and pharmacological reactions. OOAC is widely used in biological defense tactics and precision medicine. The chip is a microfluidic device that can guide and manipulate minuscule quantities of solution, ranging from pico-liters to milliliters, through a network of incredibly small microchannels. The term "organ" is more appropriate since it describes the microscopic tissues that are generated and housed in the microfluidic chips. These tissues have the ability to replicate one or more tissue-specific functions. Scientists have found that, although being far less complex than native tissues and organs, these systems may frequently function as accurate models of human physiology and illness. Organ-on-chips (OoCs) are made possible by cutting-edge in vitro technology that allows for the study of biological tissues and cells outside of the body. This is accomplished by keeping them in containers designed to maintain a biochemical and physical environment that is reasonably close to that of an in-vivo environment. This article includes the ideas behind OOAC and how it is used in drug development, physiological model building and toxicity from the viewpoint of several organs.

Keywords: Organ on chip, Microfluidic cell, Stem cell on chip, Heart on chip, Lung on chip, Brain on chip.

Introduction:

Organ-on-chip (OoC) is a relatively new tool in the toolbox of model biological systems that life scientists can use to investigate various facets of human pathophysiology and illness. These systems span a range of physiological significance, with 2D cell cultures being the least relevant and 3D cell cultures, organoids and OoCs following in increasing order of relevance. It should come as no surprise that using model organisms like Drosophila and mice allows for more physiological insight than synthetic tissue approaches.[3, 4] In model organisms, biological complexity rises with physiological significance; sadly, this

also raises the difficulty of the experiments. Despite significant advancements in in vivo imaging, in many ways the least accessible physiological processes are those that occur in mice, humans, and other animals.

However, in order to make experimentation easier, some characteristics of in-vivo relevance are sacrificed in 2D and 3D cell cultures, such as spheroids and stem cell-derived organoids. The OoC can be thought of as a bridging technology because it allows for the use of intricate cell cultures and offers better-designed microenvironments to optimize the model. Research in the disciplines of OoC and microphysiological systems has expanded rapidly since the introduction of early concepts such as animal-on-a-chip, body-on-a-chip, and breathing lung-on-a-chip. This is demonstrated by the many outstanding reviews that have been published recently. [1, 2]

OoC technology is increasingly widely recognized outside of academic labs due to the desire to discover novel ways to enhance human welfare and get a deeper understanding of the human physiology underlying health and illness. The OoC, for example, was chosen by the World Economic Forum as one of the top 2016's top ten emerging technologies.[5] This suggests that the pharmaceutical sector has a great need for human-like testing systems and that the OoC technologies needed to create them are quite mature. In a similar vein, as society looks for more compassionate in vitro alternatives to animal testing, the food, chemical, and cosmetic industries stand to gain tremendously from OoC technology for both production and testing. Converging breakthroughs in tissue engineering and microfabrication have been beneficial to OoC technology.

The field of cell and tissue engineering has advanced from simple 2D monocultures to intricate 3D co-culture systems. The geometrical layout and cellular microenvironment have received a lot of attention since they allow for direct cell–cell interaction [6, 7] cell polarization and the propagation of chemical and electrical signals. [8, 10] In addition to more sophisticated celllines21, primary cell sources have been handled with greater rigor and dependability [11, 12] when incorporated into artificial structures to support organ-like functions. With the ability to develop patient-specific cells from iPSCs produced from individual donors and incorporate them into the OoCs, the emergence of induced pluripotent stem cell (iPSC) technology promises personalization of OoCs [13, 14]. This enables patient-specific research on medication responses and illness phenotypes. [15]

The second factor contributing to the development of over-the-chip manufacturing (OoC) has been microsystems technology, a catch-all word encompassing fabrication techniques derived from the integrated circuit industry. This method creates structures in the nano- to micrometer range by using lithographic pattern transfer.[16] OoC development milestones coincide with significant advancements in microsystems technology utilized first to create laboratory-on-a-chip systems in analytical chemistry [17, 18] Microsystems technology has fueled the advancement of OoC sensing capabilities as well as microfluidic and tiny actuator development. This has led to a change in the design, operation, and monitoring of in-vitro bioreactors and cell biological systems. [19, 20] The conventional well plate or Petri dish's flat polystyrene (PS) surfaces are no longer present. It is now possible to see in-vitro organ function in chips that are n function by mimicking the cellular and extracellular characteristics of the organ in response specifically

tailored for the target organ. The chips are designed to mimic and maintain organ to biochemical and physical signals. OoC systems are important because they allow for multi-parametric read-outs of organ function, which open a window into the combined biology of humans and animals. OoC technologies have significantly developed and evolved, and it is anticipated that interest in them will only increase in the years to come. For individuals who are unfamiliar with the area, however, it could appear that there are just as many instances of OoCs in the literature as there are applications. Choosing where to begin might be difficult. The purpose of this primer is to present the elements of OoCs that should be taken into account when creating an experiment.

The purpose of this summary is to show how significant inventions during the last 20 years have progressed the field. The primer addresses the fundamental ideas and factors involved in designing, building, and running an Out-of-Case (OoC) in addition to the assaying methods used later on to retrieve biological data. There is also a debate about application for ooc technology.

Trial and Error on OoCs:

The purpose of organotypic cultures is to cultivate organotypic tissue in order to preserve function or to direct a group of cells toward assembling into a 3D tissue that can replicate one or more organ-level functions. Trial and error method should serve as a guide for OoC development. [21] When setting up a new experiment, the factors, tactics, and tools required for any general OoC experiment are reviewed in the order that they occur. Lastly, a number of case studies of OoCs involving single and multiple organs offer insight into the creation of these platforms for certain drug research applications.

Design and conceptualization of OoCs:

A high degree of biological authenticity is frequently attained by single-organ systems, which enables assessment of an organ's reaction to a chemical or combination of compounds. The framework offered by multi-organ systems allows for the investigation of possible interactions between one organ and at least one other, primarily through the exchange of metabolites or soluble signaling molecules. Since single- and multi- OoC systems are made to mimic aspects of animal or human biology in a microscale culture, they are frequently referred to as microphysiological systems [22]. Body-on-a-chip refers to multi-OoC systems that simulate the physiological systemic reaction in the body. Selecting between a single-organ and multi-organ system is based on the required features in order for the system to accurately simulate physiological processes. The least amount of complexity essential to accurately depict the biological application should be maintained, and no extraneous elements that would make the system challenging to operate and analyze should be added. Compared to single-organ systems, multi-organ systems often require more intricate technical designs. Control over the movement and distribution of culture media among the many organs is made possible by this. Thus, single-OoCs are more biologically accurate models of an organ, whereas multi-OoCs use less accurate organ models and concentrate on the systemic interactions across organs. [23] This has led to a trend in the OoC systems that have been developed. Selecting a method for creating functioning tissues inside the OoC is the next design factor to take into account.

An artificial tissue, like a pre-made organoid, or a primary tissue, such an organ slice from a biopsy, are integrated into the OoC system in a top-down or organotypic way. Using a bottom-up methodology, singled-out cells from primary immortalized lines or sources of stem cells are cultivated in an a prior empty microfluidic medium, which facilitates the cells' remodeling into a functioning neo-tissue. The OoC architectures are designed with the chosen strategy in mind. This has two purposes:

- It routes fluids, including the culture medium, to connect tissue components in the OoC.
- Supports and arranges cells in a particular cell culture configuration in a way that mirrors their in-vivo connectivity.

OoC device architectures come in a wide variety of forms, however they can be broadly categorized into two groups according to the organ systems they build.

1. The first consists of solid organ chips, in which cells are grown as three-dimensional tissue masses with the ability to communicate with one another and the culture medium in a pre-determined way. Micro-pillar and microwell arrays, which are frequently employed in the liver, tumor, heart and adipose OoCs, are examples of this sort of architecture. [24, 25]
2. The second category includes barrier tissue chips, in which the architecture of the device encourages the cells to naturally create a barrier between fluid-filled sections. This makes it possible to research the processes of selective transport across the barrier. Typically, these architectures are present in the skin, lungs, and gut OoCs. [26]

The final functionality of the OoC determines the decisions made for either the architecture or the culture strategy.

Selection and manufacture of materials for OoCs:

Numerous aspects influence the choice of material, such as read-outs, biocompatibility, microfabrication process, and the final device's functionality. The final device in an OoC device is typically constructed from a variety of material combinations. Among the most widely used materials include thermoplastics like PS, poly (methyl methacrylate) (PMMA), polycarbonate (PC), and cyclic olefin copolymer (COC), silicone rubber such as poly(dimethylsiloxane) (PDMS), glass, and thermoplastics.

But as all materials have benefits and drawbacks, there is no ideal standard material. Selecting a material frequently involves striking a balance between the product's development stage, intended functionality, and availability of fabrication equipment. Although costly and requiring sophisticated processing equipment, glass is resilient and inert. Silicon also enables the creation of complex. Over the past 20 years, organ-on-a-chip (OoC) systems have become more complicated both biologically and technically, reflecting researchers' increasing quest for more detailed knowledge about biological systems.

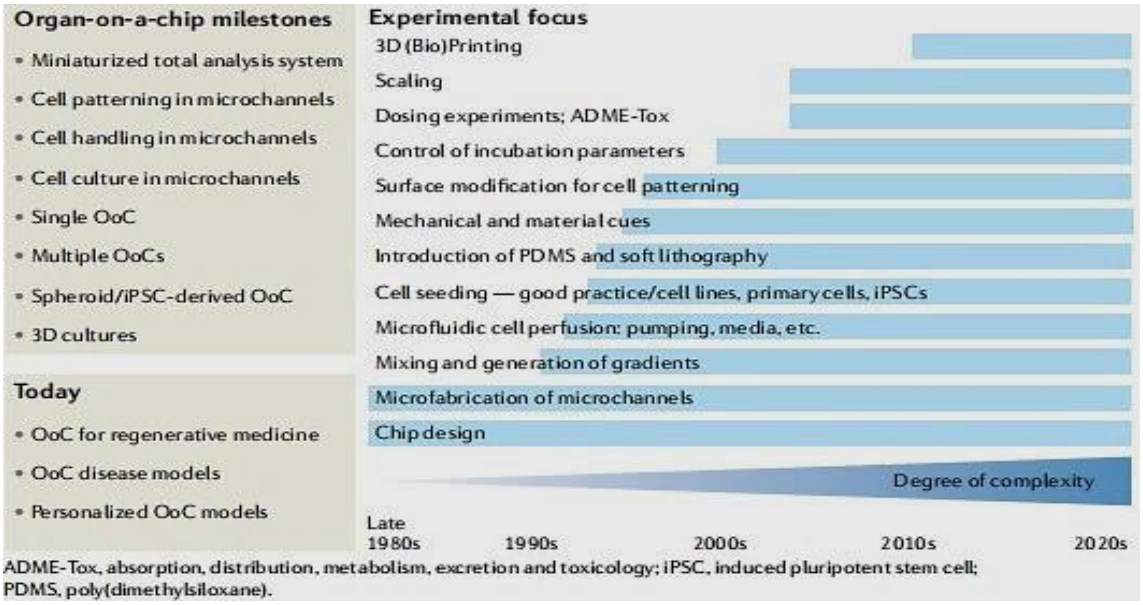

fig 1: organ-on-chip milestones [9]

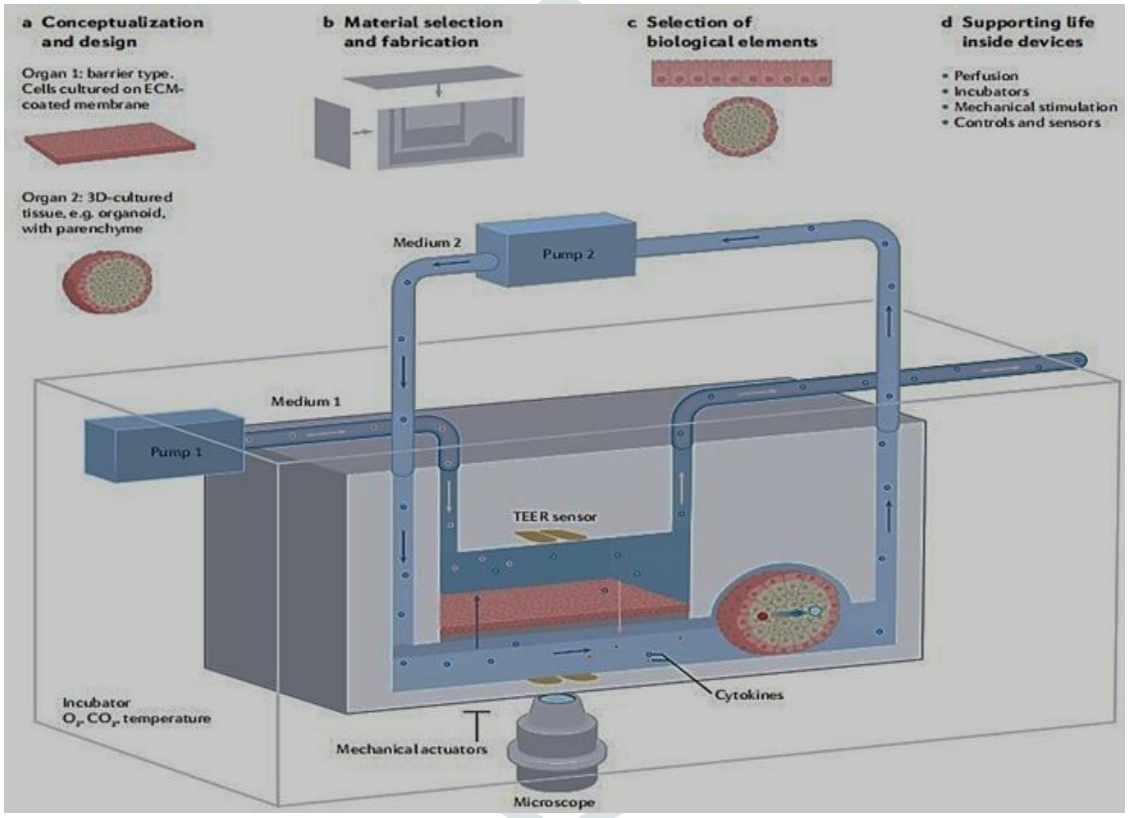

fig 2: experimental set up for a generic two organ system with supporting peripheral equipment

Thermoplastics guarantee transparency and are simple to mass-produce, but they present difficulties when developing intricate patterns during the prototyping stage. Because devices with high-resolution microstructures and nanostructures can be readily made by replica molding in PDMS from microfabricated templates, PDMS is now the most extensively utilized material for the creation of OoC devices. Because of its optical transparency, gas permeability and biocompatibility, PDMS is perfect for biological applications. Moreover, mechanical stimulation of cells has been applied using PDMS's elasticity. Newly developed technology has been offered that allows for high-quality and high-speed PDMS.[27] But its known that this substance may both adsorb and absorb a variety of (bio)chemicals, which could affect the

outcome of an experiment, particularly in applications involving drug testing.[28]

One of the substitutes frequently seen in newly created OoCs as well as conventional cell culture instruments like tissue culture flasks and multi-well plates is PS. Notably, production yield—one of the challenges facing the current OoC fabrication method—can be addressed by using injection molding for mass production, while micro-milling is used for single prototypes. This productivity may offer a path toward effective out-of-cell (OoC)-based high-throughput screening, which enables automated testing of several therapeutic compounds against a particular biological target. For out-of-the-box models with intricate patterns and practical features like stretching, injection molding is not the best option. Thus, the manufacturing process ought to be chosen with consideration for the goal of the experiment. As additive manufacturing, also known as 3D printing, develops, several organizations have reported OoC models produced by this process. [29] With this technology, complicated 3D structures that have proven challenging to produce with the other methods outlined can be created rapidly and accurately. Currently, the lack of sufficient optical transparency in 3D-printed microfluidic devices for out-of-cell applications is a limitation caused by unoptimized resin formulations and post-processing procedures. Furthermore, it is necessary to confirm the biocompatibility of 3D-printed resins.

However, OoC technology has advanced to the point where materials chosen for experimental objectives can be used to adopt efficient production techniques. Numerous alternatives have been put up, such as the creation of intricate structures using PDMS-based soft lithography to create etched microchannel arrangement and 3D printing and injection molding for large manufacture of OoC based on plastic.

Sterilisation of OoCs:

As a prior review pointed out, OoC devices are distinct from traditional cell culture platforms (such as multi-well plates) because of their 3D structures and the materials they are made of. Nonetheless, the requirements for conducting cell culture in out-of-cell devices are comparable to those of conventional platforms. [30]

Therefore, it is necessary to guarantee the sterility of the OoC devices in order to prevent microbial contamination, even in the various microfluidic components that will be utilized to assemble the OoC system as a whole. The different materials used in OoC devices and microfluidic components necessitate extra care when selecting the right sterilizing techniques in order to guard against component damage. Sterilization techniques used incorrectly may harm OoC devices and microfluidic components, leading to unintended leaks during system construction. OoC devices differ from conventional cell culture platforms (like multi-well plates) due to their three-dimensional structures and the materials they are composed of, as was mentioned in a previous review. [30] However, the conditions for carrying out cell culture in out-of-cell apparatuses are similar to those on traditional platforms.

Thus, to avoid microbial contamination even in the several microfluidic components that will be used to create the OoC system overall, it is imperative to ensure the sterility of the OoC devices. In order to prevent component damage, extra caution must be used while choosing the proper sterilizing processes due to the various materials used in OoC devices and microfluidic components. Inappropriate use of

sterilization procedures can damage OoC devices and microfluidic components, resulting in accidental leaks during system construction.

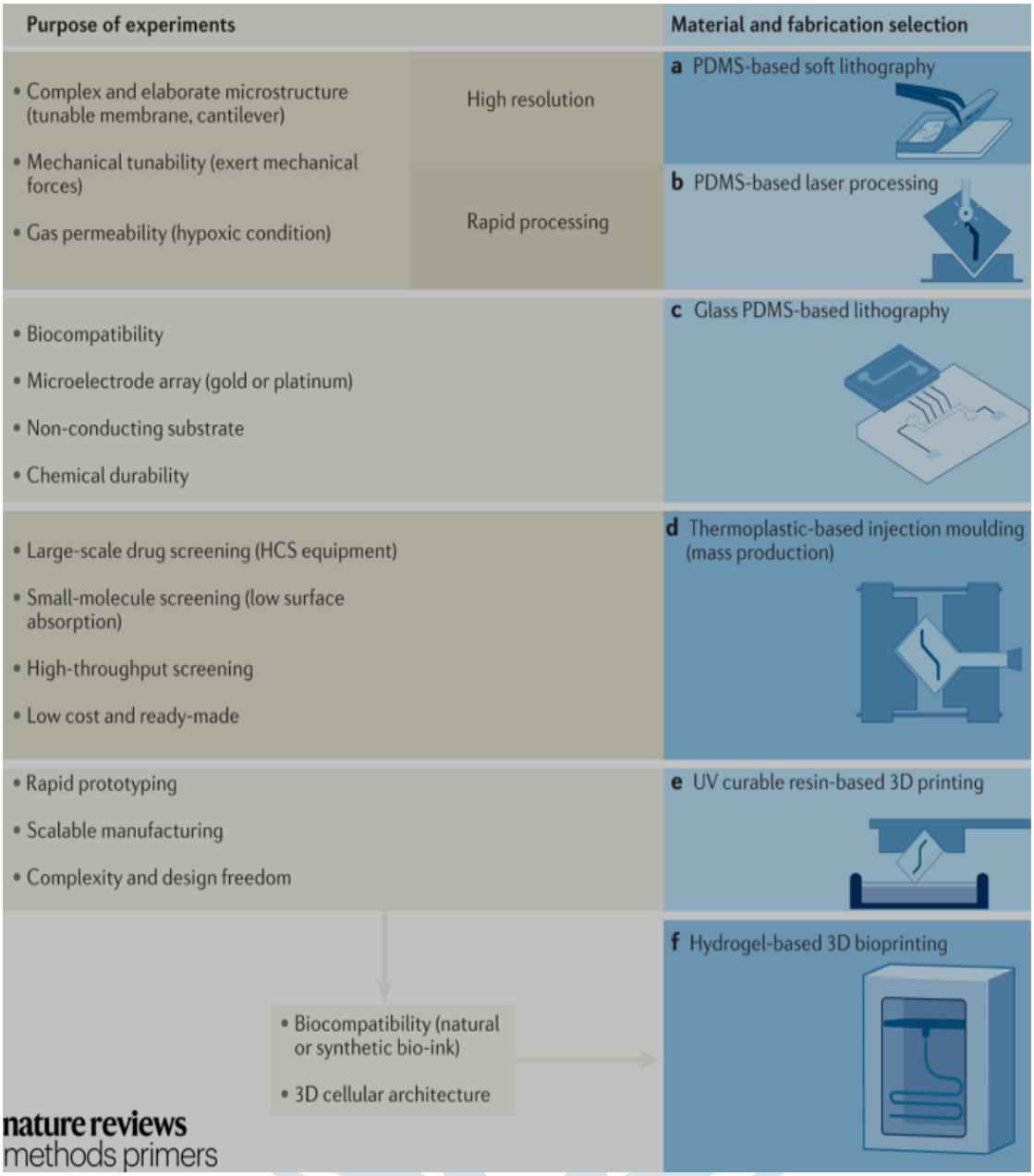

fig 3: biocompatibility and 3D cellular architecture [41]

Selection of biological elements of OoCs:

The selection of isolated cells is the main topic of discussion here because it is more frequently used in out-of-cell situations. When choosing a suitable cell source, there are a number of factors to take into account in relation to the OoC's application.

A. Internal operations of the cells:

OoCs frequently have to mimic physiological activities particular to a given tissue that are necessary for the intended use for which they are designed, such as the liver's metabolism of xenobiotics, the intestines' and skin's barrier functions and the contractility of the heart and skeletal muscles. In comparison to primordial cells, many immortalized cell lines and cells generated from human pluripotent stem cells frequently express a restricted subset or fraction of their functional capacity [31]. Primary cells may have all of the cellular functions that are present in-vivo, but when they are kept in vitro, they frequently lose their tissue-specific functions quickly. This can reduce the amount of time that the OoC is usable for, particularly for long-term investigations. Inter-donor variability in primary cells can be problematic for data reproducibility when patient-specific responses are not being examined. Consequently, it is useful to rank the various cell functionalities according to the anticipated use of the OoC while evaluating various cell sources.

B. Maintaining life within the apparatus:

1. Choosing medium for cell culture:

The culture medium that was developed for conventional cultures can be used for a single-OoC involving just one type of cell. Selecting the right media becomes more complicated when creating single or many organisms of interest (OoCs) with several cell types involved, each with unique nutrient needs. Each unique cell population's survival and functional characteristics must be preserved using the ideal co-culture medium. [32]

When this requirement is met, we can move on to evaluating the medium's viability for facilitating downstream tests without causing unwanted interference. Numerous groups have utilized different combinations of the original culture medium employed for each distinct cell type in the optimization of an appropriate co-culture medium for out-of-cell applications, with comparatively positive results.

A single-OoC involving only one type of cell can be cultured using a culture media similar to that which was created for conventional cultures. When one or more organisms of interest (OoCs) are created, choosing the appropriate media becomes more difficult because each cell type has different nutritional requirements. The optimal co-culture medium must be used to maintain the survival and functional properties of each distinct cell population. Upon fulfillment of this prerequisite, we shall proceed to assess the feasibility of the medium in enabling subsequent tests while avoiding undesired disruption. With relatively good outcomes, many organizations have optimized a suitable co-culture medium for out-of-cell applications by combining multiple versions of the original culture medium used for each unique cell type. OoC devices that resemble the liver and skin demonstrate how to maintain compartmentalization while allowing paracrine interactions between the compartments. Since sera are a significant source of variance and might impede tests, serum-free medium should be taken into consideration if at all possible. [33]

When conducting experiments using the OoC, media should be optimized. For non-OoC work using the same cells, the most popular cell culture medium should be used first, such as endothelial cell growth medium (ECGM) for endothelial cells or minimal essential medium (MEM)-based medium for epithelial cell cultures.

2. Perfusion circuit formation:

One of the most distinctive features of OoC devices is media perfusion, which acts as a mimic for the circulatory system and preserves a concentration gradient for convective transfer of waste and nutrients. Different pumps have been modified for OoC applications in order to propel media perfusion via the OoC device. These include peristaltic pumps, microvalve-driven actuator pumps and traditional syringe pumps. As an alternative, a number of organizations have chosen pump-free systems relying on hydrostatic pressure to drive perfusion. Whether the culture uses are circulatory flow configuration or a one-pass type of perfusion flow will have a significant impact on the choice of pumps.

One-pass perfusion flows are usually supported by conventional syringe pumps and pump-free gravity driven flows, although peristaltic pumps and other pump types can be used to drive re-circulatory flow. But in recent times, OoC devices with gravity-driven re-circulatory flows have become more common. This reduces the complexity of the system overall and nevertheless replicates the soluble factor crosstalk between different organ compartments. The requirements of the organ model and the in-vivo environment, including the proper degree of shear stress produced by flow across a tissue surface, determine the option for either kind of flow and the flow rate (volume per time). Although peristaltic pumps and other pump types can be utilized to generate re-circulatory flow, conventional syringe pumps and pump-free gravity-driven flows are typically employed to provide one-pass perfusion flows. However, OoC devices that use gravity-driven re-circulatory flows have proliferated recently. This duplicates the soluble factor interaction across several organ compartments while also simplifying the system as a whole.

The choice of type of flow and the flow rate (volume per time) are determined by the needs of the organ model and the in vivo environment, including the appropriate degree of shear stress generated by flow across a tissue surface. Thus, even with full replacement, only a little amount of sample (less than 100µl of medium every day) can be removed. In systems that simulate realistic fluid to cell ratios, samples as small as 25–50µl per day may be provided for offline analysis because partial fluid refilling is favored to maintain more physiological conditions. Transferring conditioned media sequentially from one single-OoC (the liver) to another single-OoC (the kidney) is one technique used to drive organ OoC interactions in addition to pump/gravity-driven perfusion flows. The perfusion techniques mentioned above that involve direct interactions between OoC modules are referred to as physical coupling of OoCs, whereas this is known as functional coupling of OoCs. The technical difficulty of adding pumps or other mechanisms for generating gravity-driven flow, as well as the requirement for connections between various OoC modules, are eliminated via functional coupling.

In addition to pump/gravity-driven perfusion flows, one method utilized to induce organ OoC interactions is the sequential transfer of conditioned media from one single-OoC (the liver) to another single-OoC (the kidney). While this is recognized as functional coupling of OoCs, the perfusion approaches discussed above that entail direct contacts between OoC modules are referred to as physical coupling of OoCs. Functional coupling eliminates the need for connections between different OoC modules and the technical challenge of adding pumps or other devices for producing gravity-driven flow.

C. Regulation of the cellular milieu:

Cells interact in a three-dimensional milieu with distinct physico-chemical properties with soluble substances, the extracellular matrix, and adjacent cells. All of this is referred to as the cell microenvironment, and it needs to be carefully regulated in the OoC system in order for cells to perform as intended.

OoC systems provide more precise control over the cell microenvironment than conventional bulk cell culture systems because the culture chamber's geometries and the related physical and chemical phenomena of the fluids are defined with micro-scale resolution (in the order of 101–102 μm). Fundamental distinctions exist between conventional static cell cultures and microfluidic-based cell cultures due to the distinct physics of microfluidics. The micro-scale volumes and material selections of OoCs, worsen common phenomena seen in conventional macro-scale cell cultures, like bubble formation, evaporation, and nutrient depletion and they can significantly change osmolality, p^H and nutrient availability. Peclet number (Pe; convective/diffusive transport), Damkohler number (Da; diffusion/reaction timescales), and Reynolds number (Re; inertial/viscous forces) are dimensionless numbers that express the relative dominance of competing physical phenomena that are relevant to micro-environmental control.

Since the mass transport regime of soluble components and fluid shear stress, for instance, are both dependent on flow velocity, adjusting an OoC's perfusion flow rate can simultaneously affect the soluble signaling and the amplitude of the shear stress. In order to selectively maintain one environmental component constant (at least from the biological standpoint that there are minimal effects on cells) while modifying another, dimensionless numbers can be utilized to direct device size and operating settings. For example, the Pe can be used to make sure that every cell is in the convective mass transport zone (Pe>1) while using a multiplexed device to investigate the effects of various shear stress magnitudes on cells. This would be beneficial. On the other hand operating at flow rates where all cells are subjected to shear stresses below a certain threshold may be advantageous when attempting to investigate the effects of secreted autocrine or paracrine substances. Preventing adverse effects on shear-sensitive cells is especially crucial, as these include embryonic stem cells ($<10^{-3}$ dynes cm^{-2}), primary neurons ($<10^{-3}$ dynes cm^{-2}), and hepatocytes ($<10^{-1}$ dynes cm^{-2}), among others.

It is important to make sure that the culture medium is properly equilibrated with the gaseous environment. The preservation of an appropriate oxygen concentration and physiological p^H (7.0–7.4) depends on the gas composition of the air. Oxygen can be maintained at atmospheric levels (21%), reduced or eliminated to replicate hypoxia (<10%) 10^2 in anaerobic systems, or raised to enhance tissue section oxygenation, depending on the requirements of the OoC. The majority of standard cell culture media, like RPMI 1640 or DMEM, are made using bicarbonate buffers, which only produce a physiological p^H of 7.0–7.4 when adjusted with 5% carbondioxide. Hence, standard cell culture incubators usually employ 5% carbon dioxide. If the OoC is used anywhere other than traditional incubator, as on a transparent indium-tin plate or heating block, prior to being injected into the OoC device. Compared to cells grown on tissue culture polymers in conventional cell culture, cells generated in OoCs may have a distinct baseline function. As a result, caution must be used when attributing variations between OoCs and traditional cell culture to the particular environmental cue that the microfluidic device is applying.

New innovations in OOAC:

Liver OOAC: The primary location of drug and toxin metabolism is the hepatic system. The complex hepatic lobules that make up the liver allow for multicellular functional communication. [34] It is difficult to keep hepatocytes functioning normally for a long time. [35] The first liver-based system was created by Kane et al., and included microfluidic holes where rat-liver cells and 3T3-J2 fibroblasts were co-cultured to resemble an airway interface.

Cultured rat hepatocytes in the chip were able to metabolize and manufacture albumin continuously and steadily. In order to replicate the interstitial structure of endothelial cells and cultured primary hepatocytes, Lee et al., created a chip that had culture media perfused outside the gap.

The hepatic system is the main site of drug and toxin metabolism. Multicellular functional communication is enabled by the liver's complex hepatic lobules. [34] Long-term maintenance of normal hepatocyte function is challenging. [35] Kane et al., developed the first liver-based system, which had microfluidic holes in which 3T3-J2 fibroblasts and rat liver cells were co-cultured to mimic an airway interface. The chip's cultured rat hepatocytes were able to consistently and continuously metabolize and produce albumin. Lee et al., developed a chip with culture media perfused outside the gap to mimic the interstitial structure of endothelial cells and cultivated primary hepatocytes. The primary location of drug and toxin metabolism is the hepatic system. Hepatic lobules are complex structures in the liver that facilitate multicellular functional communication. [34] It is difficult to maintain normal hepatocyte activity over the long term. [35]

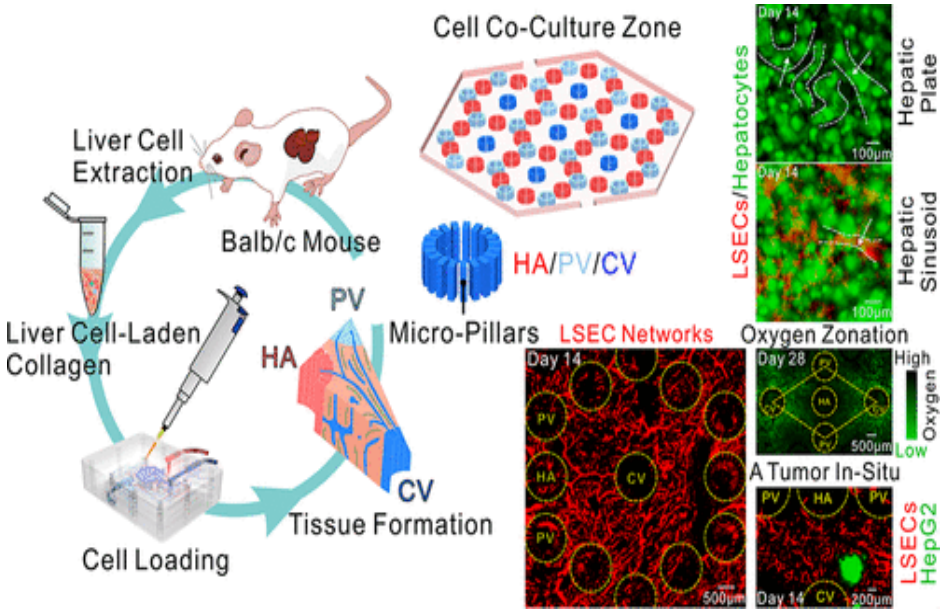

fig 4: liver on a chip [42]

Breathe on a chip:

The alveoli, which control gas exchange in the lungs, are difficult to replicate in-vitro. Microfluidics, by means of precise faid flow and prolonged gas exchange, can create extracorporeal lung models and lung panels. The blood–blood barrier (BBB), the control of airway mechanical pressure, and the impact of shear force on pathophysiological processes have been the main topics of current research [36]. Using soft lithography, Huh et al., created a lung-on-a-chip model by dividing the chip into areas separated by extracellular matrix (ECM)-containing 10 μm PDMS membranes. Human pulmonary microvascular endothelial cells were found in the lower PDMS sections, emulating the alveolar–capillary barrier, whereas alveolar epithelial cells were seen in the top parts.

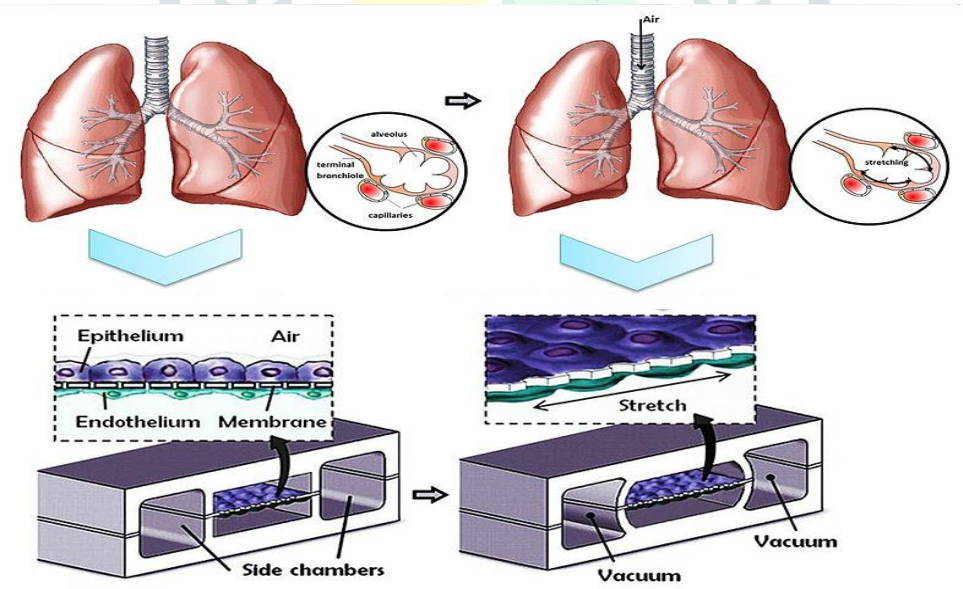

fig 5: lung on a chip (breath on chip) [43]

An imitation lung chip that resembled the lung parenchyma was described by Stucki et al., in 2015. The system was the first elastic membrane expansion model to replicate breathing and incorporated an alveolar barrier and 3D cyclic strain that simulated respiration. By exchanging both fuid and media, Blume et al.,

created 3D airway culture models that replicated pulmonary interstitial flow. [37] This made it possible to do more thorough physiological research on the epithelial barrier. This model combines many chambers for enhanced integration and uses a stent with a permeable filter as a single tissue culture chamber. In the lung-on-a-chip, pressure can be applied to the alveoli and associated capillaries, generating a shear flow profile, while replicating lung gas–liquid interfaces and respiratory dilation through the microfluidic system.

This accurately mimics the environment of the lung. In order to determine whether smooth muscle and airway epithelial cells might be used as a physiological model, Humayun et al., cultivated the cells on opposite sides of a hydrogel membrane. The system was used as a physiological model of chronic lung illness in conjunction with environmental cues and toxin exposure. A poly (lactic-coglycolic acid) (PLGA) electrospinning nanofiber membrane was created by Yang et al., to serve as a chip matrix for cell scaffolds. The system's simplicity makes it suitable for tissue engineering techniques and lung tumor precision therapy.

It is possible to use lung tissue organ chips as implanted respiratory support systems. Lung assist devices (LAD) were developed by Peng et al., to allow premature newborns experiencing respiratory insufficiency to have extra gas exchange in the placenta. The umbilical arteries and veins were found to have large-diameter channels, which allowed LAD to have a significant extra-corporeal blood flow. Due to unethical clinical trials for umbilical vasodilation thresholds, this has additional utility. This study was the first to quantify damage to the umbilical vessels caused by catheter extension in a systematic manner. Double-sided gas distribution was used in the microfabrication of micro-fluidic blood oxygenators by Dabaghi et al., in order to enhance gas exchange. In contrast, oxygen intake rose to 343% to equipment that are solitary. Xu et al., evaluated various chemotherapy medications using a microfluidic chip platform to replicate the microenvironment of lung cancer using primary cancer cells and cancer cell lines. In a different recent work, a "small airway-on-a-chip" model was used to simulate asthma. Therapeutics were examined using human airway models for asthma and chronic obstructive pulmonary disease, and the chip model replicated in vivo responses to a comparable medication.

Kidney OOAC:

The kidneys are in charge of osmotic pressure regulation and medication excretion. The irreversible loss of renal function caused by kidney toxicity emphasizes the necessity of drug screening programs. The glomerulus, renal tubule, and renal capsule make up the nephrons, which are the sites of reabsorption and filtering. Microfluidics offers porous membrane support for the preservation of cell polarity and can mimic the fluid environment that promotes tubular cell development. The first multi-layered microfluidic was created by Jang et al., using mouse kidney medullary collecting duct cells to mimic renal failure. The gadget offered a biomimetic setting that, in response to hormone activation, promoted cytoskeletal remodeling and molecular transport, therefore improving the polarity of the inner medullary collecting duct. 2013 saw the cultivation of human primary renal epithelial cells using the same microfluidic apparatus. These were the first investigations on the toxicity of primary kidney cells. This tool makes it possible to directly visualize and quantitatively analyze a variety of biological processes occurring within the intact kidney tubule been

feasible in conventional cell culture or animal models, and it might also be useful for researching the fundamental molecular pathways underlying renal illness and function.

Extended culture durations and an external signal monitoring system are necessary for cell differentiation into functional cells, which is a drawback of conventional cell culture systems.

In organ culture devices, Musah et al., reported how to induce human glomerular chips from podocytes obtained from pluripotent stem cells. With these, the structure and functionality of the glomerular capillary wall could be replicated, something that was not achievable with other techniques. The chip was useful for kidney development and disease, regenerative medicine, pharmaceutical development, and nephrotoxicity studies.

In human proximal tubules and glomeruli, Sakolish et al., created a reusable microfluidic chip that allowed renal epithelial cells to proliferate in a variety of settings. Nephrotoxicity is caused by shear stress. Stable tubule culture systems that allowed for prolonged expansion and the examination of human kidney tissue were created by Schutgens et al. A versatile primary renal epithelial cell culture model was created using the technology, allowing for quick and customized molecular and cellular investigation, illness modeling, and medication screening. A potent method for creating human islet organoids from human induced pluripotent stem cells was described by Tao et al. This approach was useful for a variety of regenerative medicine and stem cell-based organic engineering applications.

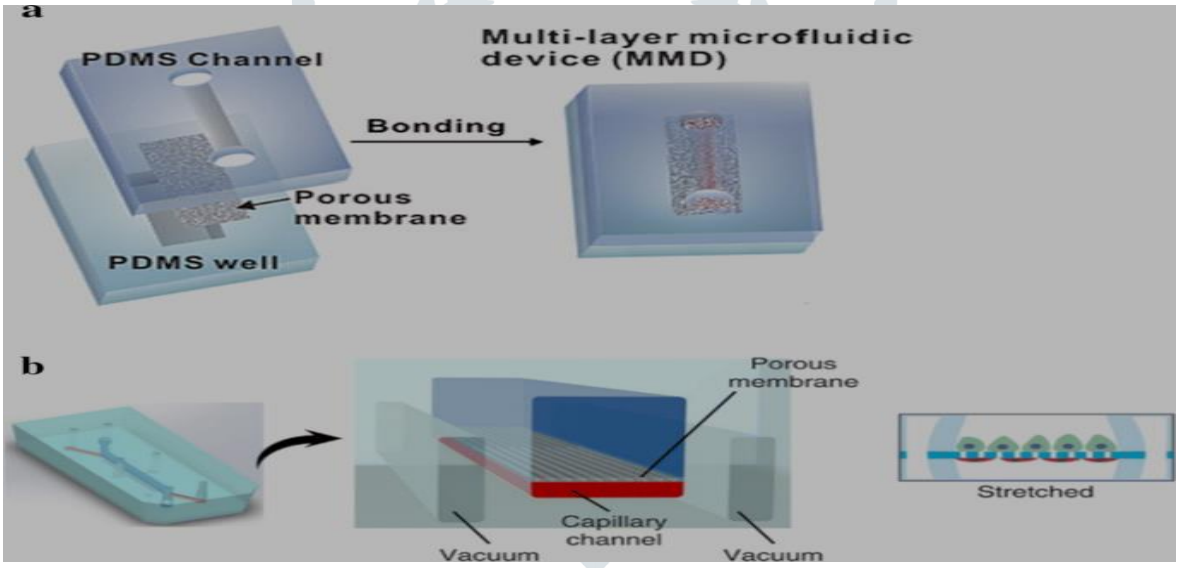

fig 6: kidney on a chip [44]

Heart-on-a-chip:

The primary cause of death for people is cardiovascular disease. The development of microfluidics has made it possible to study heart tissue in-vitro using bionics. One of the heart's main components is the myocardium. Cardiomyocyte (CM) beating is directly correlated with heart pumping and can be utilized to measure the effects of drugs. Grosberg et al., created an elastic film with a surface texture in 2012 using PDMS, and then they implanted neonatal rat CMs on the membrane to create muscle membranes. [38] The muscle flexed to one side when the CMs contracted. It was feasible to examine the variations in the sizes of

the cell contractile capabilities on the PDMS film by calculating the degree of this curl.

The experimental setup proved appropriate for high-throughput automated multi-plate tests as well as measurements of individual muscle membranes. Later, in a PDMS model, Zhang et al., created self-assembling cardiac sheets using hydrogels in 2013. The differentiated myocardium served as the source of the CMs. With the use of 3D printing technology, micro-organ tissue chips were created that allowed the vascular and cardiac systems to be integrated. Vascular endothelial cells were used in the model to create vascular networks, and CMs were introduced to fill in the spaces left by these networks. The organ chip created a drug screening platform for cardiovascular diseases. The heart-on-a-chip device, which evaluated the effectiveness of cardiac drugs using high-speed impedance sensing, was first shown by Zhang et al. The instrument shows the effects of the medicine by recording the contraction of the CMs. The medication's cardiac efficacy was evaluated pre-clinically using the chip. A heart organ platform that replicated the mechanical and physiological conditions of CMs was constructed by Marsano et al. It was possible to do quantitative analysis and direct visualization, which were not possible in conventional cell culture or animal models. This platform offers standard functional 3D heart models and is an advancement in the field. This makes the gadget a cutting-edge, reasonably priced screening tool to increase the accuracy of in-vitro models. Schneider created chips that are practical and effective for producing cardiac tissue in a regulated setting using human-induced pluripotent stem cells. The cardiac tissue remained viable and functional over an extended duration, and precise spatiotemporal pulsation dynamics were seen by optical means. Numerous biomedical applications can make use of this platform. Furthermore, according to Tzatzalos et al., this PSC-CMs can represent an infinite potential for both disease-specific and healthy CMs to evaluate the effectiveness of medications for dilated cardiomyopathy. The discovery of new medications has significant consequences for cardiovascular tissue because cardiotoxicity, which is frequently observed during drug trials and is a primary cause of clinical trial suspensions or drug recalls, affects cardiovascular tissue.

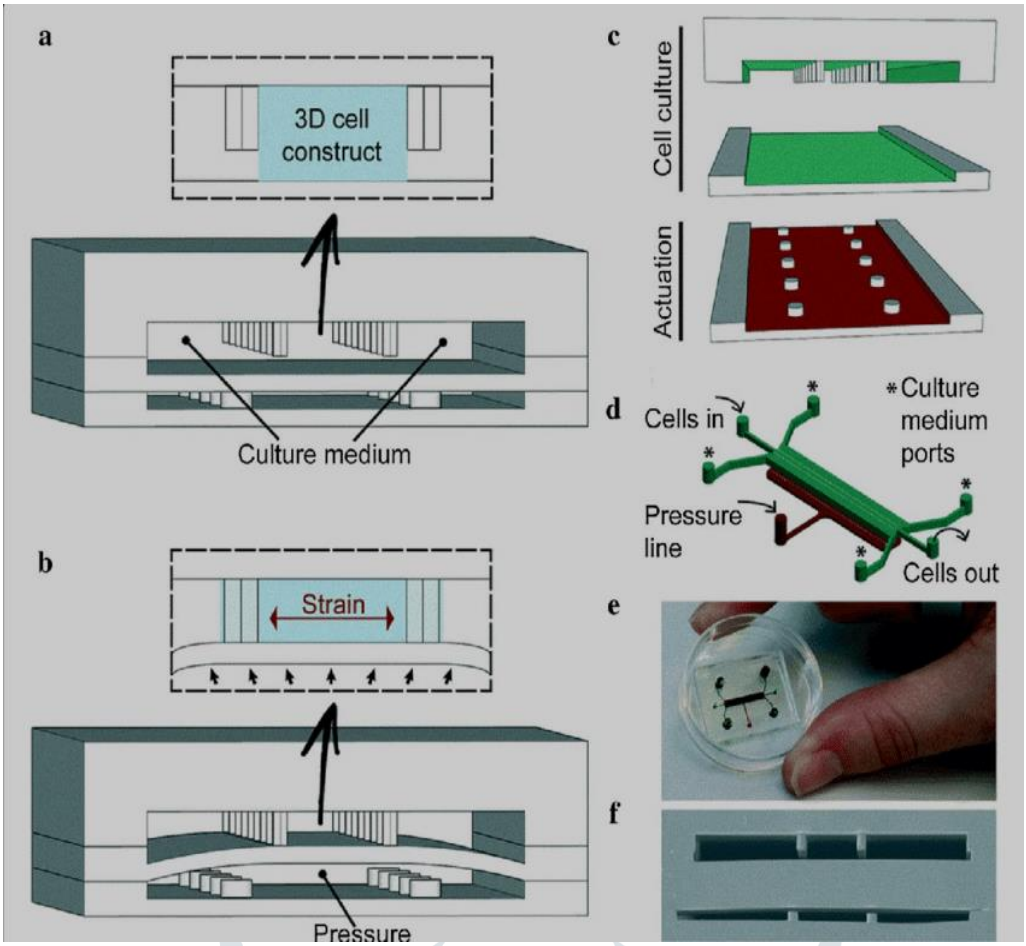

fig 7: 3D heart on a chip [45]

Several organs on a chip:

Interactions between tissues and constant media circulation are necessary for a variety of physiological pathways. The intricacy, functional variations, and integrity of organ function are not adequately reflected by single organ chips. The "multi-organ-on-a-chip," also called the "human-on-a-chip" is a device that concurrently builds several organs, garnering significant interest from researchers. In order to accomplish multi-organ integration, multi-organs-on-a-chip cultivate cells of many organs and tissues simultaneously that are connected via channels (bionic blood vessel allowing the investigation of interactions to construct a system. These can be divided into three categories: flexible, semi-static, and static. Static organs are combined into a single, networked device. The organs in semi-static systems are connected via fluidic networks using tissue inserts based on Transwell® Individual organ-specific platforms in the flexible system are connected by flexible microchannels. The flexible nature of these systems is advantageous as it recreates multiple organs. Major advancements have been made in the design of two-organs, three-organs, four-organs and ten-organs on a chip despite the fact that the concept of multi-organs-on-a-chip is still in its infancy.

The first person to mix the liver and intestines in a microfluidic device was Van et al., (2010). The liver and intestinal slices worked on the chip and showed how it may be used to study organ relationships, such as the control of bile acid synthesis. This technique allowed for in-vitro research and shed light on the relationships between different organs. Since then, a greater variety of organs have been arranged on a single chip. To ensure a strong filament attachment, prevent bacterial contamination, and track the survival of the cells during the culture phase, organ chips are necessary. Unpredictable outcomes are inevitable as the

system's complexity rises with the number of organs on the chip. Expanding the scope of applications requires simplifying current systems.

Pumpless, user-friendly multi-organs-on-a-chip that was simple to assemble and operate was created by Lee et al. A multi-throughput multi-organ-on-a-chip system based on a microplate-sized pneumatic pressure-driven medium circulation platform was described by Satoh et al. This system has the following benefits for use in drug discovery: it can operate multiple multi-organ culture units simultaneously; it can be applied to widely used for analytical techniques and experimental protocols in microplates; it has a pipette-friendly liquid handling interface; and the microfluidic network's design is flexible. This multi-organ culture platform will be a useful drug discovery research tool. It was necessary for the OOAC to continue developing if design, modeling, manufacturing, and usability improved. Lantada and his colleagues created a novel fusion of laser technologies. The evaluation using human mesenchymal stem cells confirmed the method's effectiveness, and the resulting clear chip made imaging processes easier. These technologies hold potential for mass-produced semiconductors and are useful for the transportation, aerospace, and energy sectors.

Recent years have seen a rapid development of OOAC technology, which has improved our understanding of all the major organs. Blood vessels the skin the BBB skeletal muscle, and the CNS are some other topics not covered in this study.

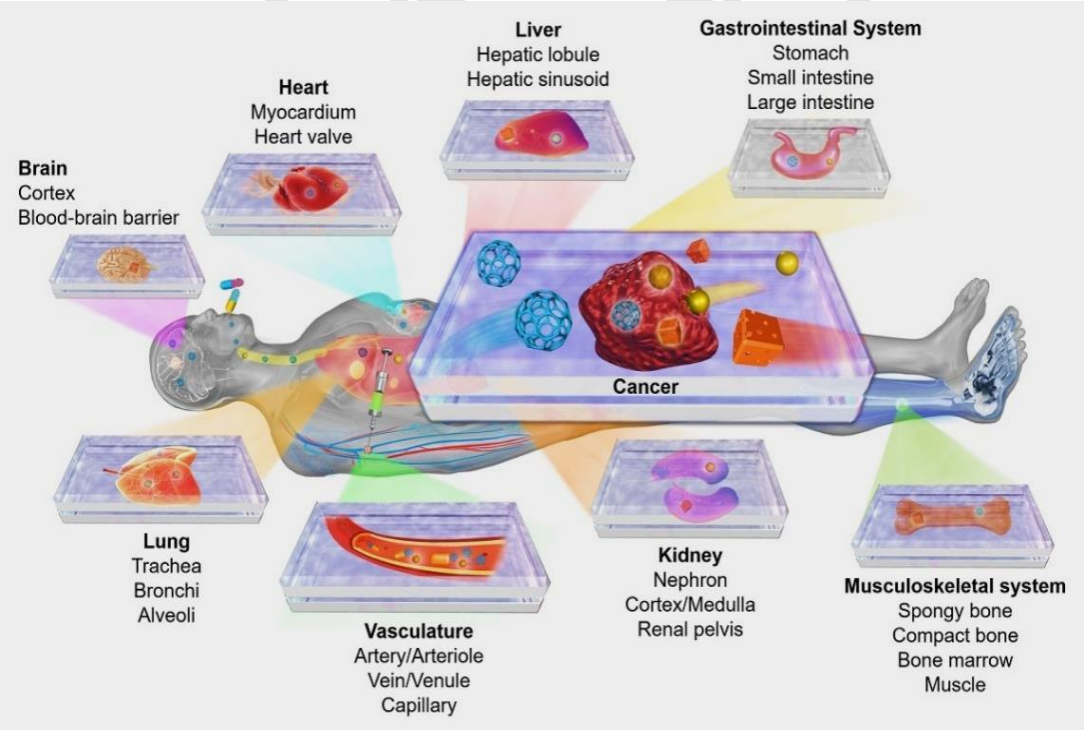

fig 8: different types of organ-on-chips [46]

Engineering with stem cells:

One of the most crucial elements in the design of an OOAC is the source of biological tissue. Human stem cells can be obtained without a tissue biopsy. Any self-renewing cell with the capacity to differentiate into one or more specialized cell types is considered a stem cell. Adult stem cells (ASCs), induced pluripotent

stem cells (iPSCs), and embryonic stem cells (ESCs) are the three most prevalent forms. As a biological tissue source for OOAC, these cells can be employed.[39] Mesenchymal stem cells (MSCs), which are pluripotent stem cells isolated from adult tissue, are the most prevalent type of ASCs in humans. Because bone marrow or adipose tissues are the usual sources of bone marrow mesenchymal stem (bMSC) cells, these cells are an appealing alternative.

Compared to their pluripotent counterparts, MSCs are less helpful in OOAC models because of their restricted capacity for differentiation, inconsistent derivation techniques, and unclear biological interactions. Human embryonic stem cells, or blastocysts, are the source of human ESCs. They can differentiate into any kind of adult cell from any of the three germ layers and are pluripotent, depending on the source. Regulating and restricting human ESCs, however, is necessary because the requirement that they come from human embryos is contentious from an ethical standpoint. Clinical trials with human ESCs are more challenging due to the ethical controversy surrounding ESCs and the technological challenges of creating large numbers of genetically different cell lines than their use as precise drug substitutes in disease models for therapeutic purposes.

Like ESCs, MSCs are pluripotent and can differentiate from all three layers of germs. Since iPSCs are created from adult tissue as opposed to embryonic tissue, they are free from the moral dilemmas raised by ESCs. In cells with the same genetic background, there are no appreciable differences in the morphology, surface marker expression, or gene expression levels between ESCs and iPSCs. Apart from avoiding moral dilemmas, iPSCs have an additional benefit over ESCs: they can be derived from donors with established disease phenotypes, which makes them suitable for patient-specific disease models and medication screening.

Given their greater availability compared to several primitive cell types and tissue biopsies, as well as their greater physiological representativeness in comparison to other cell lines, stem cells are expected to take center stage as the primary source of tissue for OOAC in the future Improvements in stem cell techniques and advancements in OOAC technology will result from ongoing research into the processes by which stem cells differentiate into functioning organ models on chips. [40]

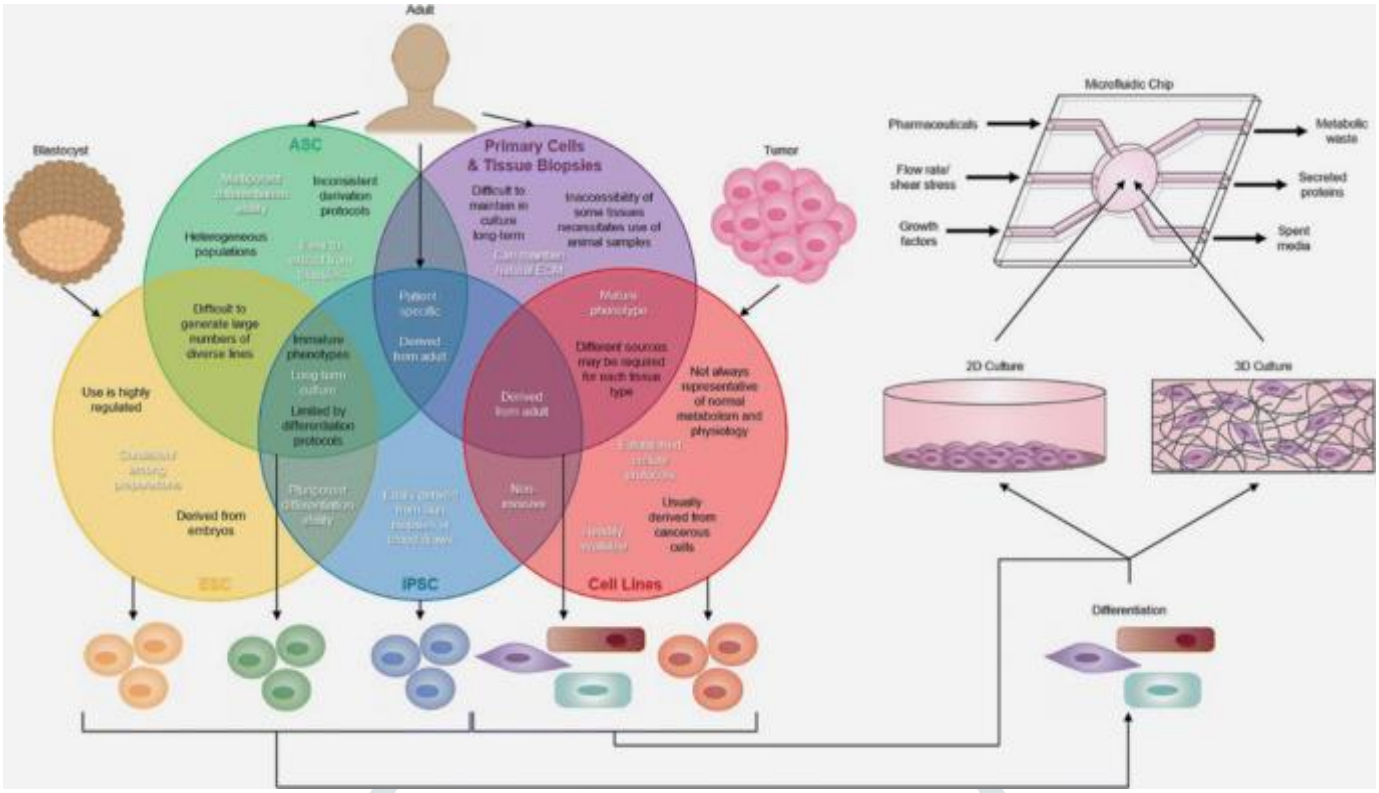

fig 9: stem cell on a chip [47]

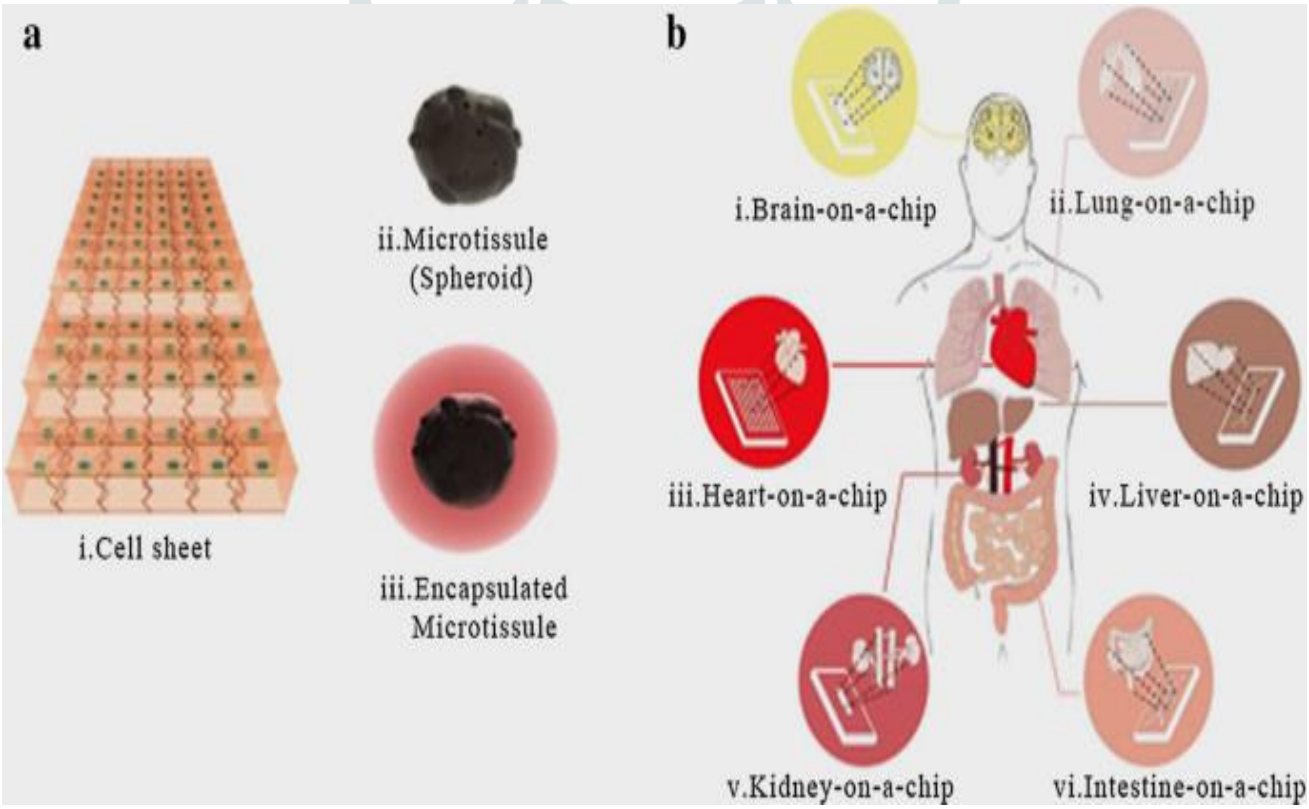

fig 10: physiology of a stem cell [48]

Conclusion:

In order to create a "Human-on-a-chip" OOAC's ultimate objective is to combine many organs onto a single chip and develop a more intricate multi-organ chip model.

Despite the quick development of OOAC technology, the idea of a human on a chip is still far off. The most commonly used polymer, PDMS, has drawbacks since the final film is thicker than the in-vivo shape. Solvent efficacy and toxicity are affected by tiny hydrophobic compounds' reduced absorbance.

Finding acceptable substitute materials is therefore essential. The cost of development and production is now high, which prevents organ chips from being widely used. As a result, parts need to be inexpensive and simple to discard. Reusable components ought to be more costly. For general use, the media volume and connector size of integrated system components must be decreased. Sample collection on the chip may disrupt its functionality and cause variations in the levels of different metabolites. Thus, more appropriate sensors are needed. All-organ compatible universal cell culture media are also necessary.

Most importantly, functionality gets increasingly sophisticated and generated data carries artifact and non-translatable dangers as the number of organs on the chip increases. This cannot currently be resolved. The indicators identified in-vitro may not entirely reflect the in-vivo equivalent in the event of repeated long-term treatment or on-chip research.

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