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FINAL DEGREE THESIS

Bachelor's Degree in Biomedical Engineering

ANALYSIS OF METHODS FOR PHYSICAL AND BIOLOGICAL CHARACTERIZATION AND VALIDATION OF MICROPHYSIOLOGICAL SYSTEMS (MPSs)

Report

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Abstract

Microphysiological systems (MPSs), also known as 'Organ-On-a-Chip' (OCC), have revolutionized the way of understanding biology. These systems, which include three-dimensional co-culture and microfluidic technology, aim to mimic human physiology with in-vitro culture systems. Their purpose is to increase the knowledge of biological processes, as well as to provide an effective diagnostic tool for the analysis of different drugs.

Standardization of MPSs implies the robustness and reproducibility of these devices and is desirable for their industrialization, production and regularization. However, due to the early stage of academic and commercial development of this technology, no standardization procedure exists in the literature to date. Therefore, experts recommend focusing on the characterization or qualification of these devices. This characterization and qualification of microphysiological systems involves testing the different elements that make up the device to ensure that their configuration mimics the physiology of the human structures represented, behaving and providing values as similar as possible to those of the tissues in vivo.

It is in this context that this thesis attempts to develop a characterization protocol applicable to any 'Organ-On-a-Chip' system based on the tests carried out and compiled in the literature of devices in the experimental or commercialization phase. Specifically, a characterization and qualification procedure is presented in which the membrane permeability is monitored in real time depending on device elements such as the presence or not of cell culture, the application or not of microfluids, among others.

The choice of the assays to be performed, from among those described in the protocol, will depend on the elements of the OCC to be characterized.

Resum

Els sistemes microfisiològics (MPSs), també coneguts com a "Organ-On-a-Chip" (OCC), han revolucionat la manera d'entendre la biologia. Aquests sistemes, que inclouen el co-cultiu tridimensional i la tecnologia microfluídica, pretenen imitar la fisiologia humana amb els sistemes de cultiu in-vitro. El seu objectiu és augmentar el coneixement dels processos biològics, així com proporcionar una eina de diagnòstic eficaç per a l'anàlisi de diferents fàrmacs.

L'estandardització de MPSs implica la robustesa i reproductibilitat d'aquests dispositius i és desitjable per a la seva industrialització, producció i regularització. No obstant això, a causa de la primera etapa del desenvolupament acadèmic i comercial d'aquesta tecnologia, no existeix cap procediment d'estandardització en la literatura fins a data d'avui. Per tant, els experts recomanen centrar-se en la caracterització o qualificació d'aquests dispositius. Aquesta caracterització i qualificació de sistemes microfisiològics implica provar els diferents elements que componen el dispositiu per assegurar que la seva configuració imiti la fisiologia de les estructures humanes representades, comportant-se i proporcionant valors el més similars possible als dels teixits in-vivo.

És en aquest context que aquesta tesi intenta desenvolupar un protocol de caracterització aplicable a qualsevol sistema "Organ-On-a-Chip" basat en les proves realitzades i compilades en la literatura de dispositius en la fase experimental o de comercialització. Concretament, es presenta un procediment de caracterització i qualificació en el qual la permeabilitat de la membrana es controla en temps real depenent dels elements del dispositiu com la presència o no del cultiu cel·lular, l'aplicació o no de microfluids, entre d'altres.

L'elecció dels assaigs a realitzar, d'entre els descrits en el protocol, dependrà dels elements de l'OCC que el caracteritzin.

Resumen

Los sistemas microfisiológicos (MPSs), también conocidos como "Organ-On-a-Chip" (OCC), han revolucionado la forma de entender la biología. Estos sistemas, que incluyen el co-cultivo tridimensional y la tecnología microfluídica, pretenden imitar la fisiología humana con sistemas de cultivo in vitro. Su finalidad es aumentar el conocimiento de los procesos biológicos, así como proporcionar una herramienta de diagnóstico eficaz para el análisis de diferentes fármacos.

La estandarización de los MPSs implica la robustez y reproducibilidad de estos dispositivos y es deseable para su industrialización, producción y regularización. Sin embargo, debido a la temprana etapa de desarrollo académico y comercial de esta tecnología, hasta la fecha no existe en la literatura ningún procedimiento de estandarización. Por ello, los expertos recomiendan centrarse en la caracterización o cualificación de estos dispositivos. Esta caracterización y cualificación de los sistemas microfisiológicos implica probar los diferentes elementos que componen el dispositivo para asegurar que su configuración imita la fisiología de las estructuras humanas representadas, comportándose y proporcionando valores lo más similares posibles a los de los tejidos in vivo.

Es en este contexto en el que esta tesis trata de desarrollar un protocolo de caracterización aplicable a cualquier sistema 'Organ-On-a-Chip' basado en las pruebas realizadas y recopiladas en la literatura de dispositivos en fase experimental o de comercialización. En concreto, se presenta un procedimiento de caracterización y cualificación en el que se monitoriza en tiempo real la permeabilidad de la membrana en función de elementos del dispositivo como la presencia o no de cultivo celular, la aplicación o no de microfluidos, entre otros.

La elección de los ensayos a realizar, de entre los descritos en el protocolo, dependerá de los elementos del OCC a caracterizar.

Appreciations

First of all I would like to thank my tutor Professor Monica Soncini for giving me the opportunity to carry out this project and the PhD candidate Alessandra Maria Anna Rando for giving me the necessary tools to fulfil it. Thanks to both of them for guiding me through the whole process and at the same time giving me the freedom of decision to develop this project.

Secondly, I would like to thank my colleagues at the Department of Electronics, Informatics and Bioengineering (DEIB) of the Politecnico di Milano, with whom I have shared many hours in the lab. It has been a very pleasant stay full of fellowship and learning. I am grateful to have been part of the research group during this period of time.

And above all, I would like to thank my mother, my father, my brother and my grandparents for the trust they have always placed in me. Thank you for helping me at all times to move forward, for being by my side during all these years and for supporting me to get this far.

Glossary

MPSs	microphysiological systems
OCC	organ-on-a-chip
ECM	extracellular matrix
PBS	phosphate buffered saline
MW	molecular weight
TEER	transepithelial electrical resistance
Micro-PIV	micro particle image velocimetry
RBCs	red blood cells
IL-2	interleukin-2
fps	frames per second
FITC-dextran	fluorescein isothiocyanate-dextran
AFA	alexa fluor
BBB	blood brain barrier
BSA	bovine serum solution
DAPI	diamidino-2-phenylindole
kDa	kilodaltons
W/h	watts per hour

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Preface

Motivation

Microphysiological systems (MPS), commonly referred to as organs-on-chips (OOC), are a category of in vitro model that are capable of simulating the physiological and mechanical characteristics of human organs and tissues [1]. MPS are microfluidic platforms with numerous microchannels and chambers that are lined with cells and tissues that replicate the particular organ or tissue under study [2]. Real-time monitoring of cellular reactions to medications and stimuli is made possible by MPS, which can offer precise control over the cellular environment.

Because MPS technology provides a more accurate simulation of human biology than conventional cell culture or animal models, it has a wide range of possible uses in the drug development process, such as drug screening, efficacy testing, and toxicity testing. Researchers can more accurately predict how medications will act in people and customize treatments for specific patients by utilizing MPS [2], resulting to more effective therapies and better results. To further comprehend the etiology of disorders and to identify fresh therapeutic targets and biomarkers, MPS can also be used in disease modeling [3]. Additionally, MPS can provide a platform for the investigation of intricate relationships between several organs, including medication toxicity and metabolism as well as the development of illness [4]. Finally, OOC has a fascinating use in personalized medicine. Doctors might tailor medicine regimens for patients to forecast potential side effects and adjust the treatment accordingly by utilizing MPS to develop a model of the individual's organ.

Even though this systems have a lot of potential for biomedical research, standardization and validation can be very difficult. One problem is the lack of a standardized design and manufacturing process for MPS, which can cause variations in their performance and dependability [2]. The intricacy of OOC also makes it difficult to validate their use. Given their dynamic nature and the number of variables they involve, traditional validation test findings like correlation coefficients or sensitivity/specificity values may not accurately represent MPS performance [3]. To determine the predictive ability of MPS, consistent validation criteria are required, and these metrics must take into consideration the variety of physiological behaviors and reactions under various stimuli and settings. To ensure the generalizability the systems results, inter-laboratory cooperation and repeatability testing are highly necessary. In this regard, the development of analytical variables and the utilization of common reference materials may be helpful. Despite these obstacles, there are ongoing academic, governmental, and business projects aimed at standardizing and validating MPS. To ensure that this technology can fully advance translational medicine, continued interdisciplinary collaboration will be required.

Requirements

Since all new concepts and technologies were learned while working on the project, no prior prerequisites were necessary for it.

Objectives

The ambition of this thesis lies in the achievement of the objectives defined in this section. This project consists of three blocks: a first theoretical part based on the compilation of documentation to understand the engineering of MPSs; a second part, also theoretical, in which the main validation tests are analyzed for different parameters of the MPSs; and a third practical part in which a universal procedure to characterize and qualify the permeability of the membrane of these systems is developed. Hence, the objectives that this project intend to achieve are:

- ➢ To know what a microphysiological system is and its applications.
- ➢ To understand how to engineer OCC systems.
- ➢ Analyze the problem of standardization and validation of these systems.
- ➢ Analyze the current standardization, validation and characterization tests of these systems.
- ➢ To create a protocol to characterize MPSs according to their constituent elements.
- ➢ Apply the protocol to a microphysiological system mimicking the exocrine pancreas.

1.

Theoretical foundations

1.1 Cell culture systems

1.1.1 Evolution of cell culture systems

Cell culture is the process of growing cells in controlled environments, usually away from their native habitat. In order to provide the necessary nutrients (amino acids, carbohydrates, vitamins, and minerals), growth factors, hormones, and gases (CO_2, O_2), an appropriate medium is needed. The environment maintains the physicochemical qualities (pH, oxygen, temperature and osmotic pressure). These components impart a particular physiological character to the environment and a collection of extracellular cues that cooperate to control cell shape, function and behavior [5]. The cell microenvironment is defined by the interaction of various biochemical, physical, and physicochemical elements and is depicted in Figure 1.

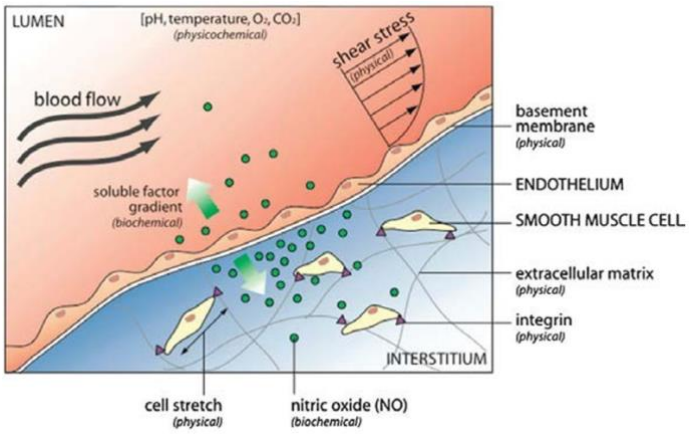

Figure 1. *Diagram showing the physical, biological, and physicochemical components that make up a cell's microenvironment [1].*

Researchers can isolate particular components for testing outside the complicated in vivo microenvironment using cell culture. By doing this, they are better able to formulate rational hypotheses about the impact of those variables and, through carefully monitored experiments, to clarify the mechanisms that drive cell activity. Cell culture has two objectives: to mimic the cellular microenvironment as precisely as possible and to keep things simple enough to provide statistically significant findings quickly. These two factors frequently have to be traded off, with model accuracy being given up for greater throughput or vice versa.

Ross Granville Harrison, a biologist and anatomist, is credited with creating artificial tissue culture. He noticed the development of nerve fibers from protoplasmic extensions in 1907 when he implanted pieces of frog embryo in hanging drops of coagulated frog lymph [6]. Harrison created a reproducible method utilizing plastic dishes, currently known as Petri dishes, to overcome the difficulties of basic culture. The techniques utilized now are strikingly comparable to those used by Harrison in 1907, despite the fact that

our understanding of molecular and cell biology has greatly advanced over time. For 2D cell culture, we continue to use undefined biological material in plastic dishes.

It has been possible to add new approaches to create new cell culture models as biochemistry, molecular biology, cell biology, and other fields of biological knowledge have advanced. There are several different in-vitro cell culture models available right now, each with a unique level of biological complexity. From straightforward 2D monolayer cell cultures of one cell type and monotypic 3D cultures to more complex models integrating numerous cell types in a more physiologically appropriate cell culture environment, in-vitro cell culture methods have progressed. These cell culture systems have been applied in a variety of research fields. They have proven crucial to the study of virology and have contributed to the reduction of the usage of experiment animals. In addition, cell culture has been utilized in pharmacology and toxicology studies to investigate the effects of various medications, drug-receptor interactions, resistance phenomena, and cytotoxicity, among other things. Tissue engineering has been an area of application that has received much attention recently.

A diagram illustrating the development of various cell culture systems is shown in Figure 2. Starting with the most basic static two-dimensional monolayers models and progressing to the more complex and dynamic three-dimensional co-cultures models. The types of cell culture systems are broken down into two categories in the following sections: static systems, which were used prior to the advent of microfluidics in cell culture systems, and dynamic systems, which used microfluidics.

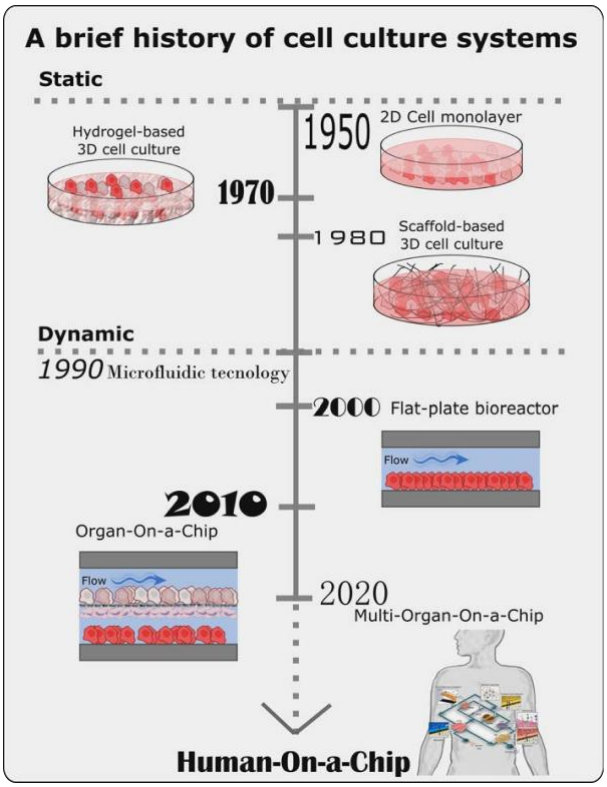

Figure 2. *Evolution over time of the cell culture systems from the simples 2D models to the complex OOC models* [7].

1.1.2 Static cell culture

1.1.2.1 Two-dimensional monolayer models

A single type of cell is adhered to and cultivated on a flat plastic or glass surface, such as micro-well plates, flaks, and Petri dishes, in typical 2D monolayer cell culture techniques. In a monolayer arrangement, the medium's nutrients and growth factors can be distributed uniformly throughout all the cells to support their growth. Necrotic cells are typically unattached from the surfaces of the monolayer and are easily eliminated during medium changes, therefore the monolayer is primarily made up of proliferating cells. While most cell types need flat surface plates for culture, others can be cultured in culture medium free floating, depending on the cell type. Since the majority of cells in the body are non-circulating, they must adhere to the extracellular matrix (ECM) in their environment in order to survive. Cells are physically attached to the ECM by means of cell-surface integrity, which is also in charge of monitoring and converting mechanical signals from focal adhesion sites to the cytoskeletal machinery inside the cell [8]. Numerous cellular functions, including as migration, proliferation, differentiation, and death, are known to be regulated by these signals.

Due to their simplicity, ease, and excellent cell viability, 2D surfaces have been used for the majority of cell culture experiments throughout the past century. Although these traditional 2D cell culture systems have significantly advanced our understanding of fundamental cell biology, they are still limited in their ability to support a wide range of investigations into the tissue-specific, differentiated functions of various cell types and the precise prediction of in vivo tissue functions and drug activities. Cells cultivated in 2D culture are typically more flat and stretchy than they would be in vivo, which is one reason for this failure.

Many biological processes, including cell proliferation, differentiation, death, and protein production, are influenced by the aberrant cell shape in 2D culture [9]. 2D cell culture systems' inherent simplicity is not always a drawback in simple cases and applications, but sadly, in complicated studies where cellular output and communication are crucial, this simplicity fails to accurately represent the entire system of interest and fails to recreate the in-vivo cellular microenvironments.

1.1.2.2 Three-dimensional hydrogels and scaffolds models

Cell-to-cell contacts are eliminated in standard 2D cell culture because when cells expand, their structure changes, causing them to flatten and deform into a basic monolayer. The creation of 3D cell culture matrices was a result of efforts to solve the drawbacks of 2D cell cultures. A significant issue in cell culture is preserving a cell's three-dimensional structure [9]. As a result of the hydrogels' linked pores' strong water retention, gases and other chemicals can be transported effectively. Animal ECM extract hydrogels, protein hydrogels, peptide hydrogels, polymer hydrogels, and wood-based nanocellulose hydrogels are

only a few of the various forms of hydrogels made from organic and synthetic materials that can be used for 3D cell culture [10].

The ECM, a complex 3D fibrous meshwork with a wide distribution of fibers and gaps that transmit complicated biochemical and physical signals, surrounds nearly all of the body's cells. These 3D cell culture matrices, often referred to as scaffolds, are porous substrates that can enable cell growth, organization, and differentiation on or inside their structure, inducing cells to polarize and interact with one another. They can appear in a variety of ways, such as cells grouped together in self-assembling cellular microstructures called organoids or randomly strewn across the ECM [11]. The capabilities of the manufacturing process being employed have a significant impact on the capacity to build scaffolds [12].

For researching the molecular underpinnings of cell function, these 3D models have been extremely helpful. These models, for instance, have shown that the topography and stiffness of the matrix influence adhesion, morphogenesis, differentiation, and viability [13][14]. They do, however, have drawbacks. Organoids come in a wide range of sizes and shapes, making it challenging to keep cells in fixed places within these frameworks for lengthy research. Another problem of these 3D models is that it is challenging to harvest cellular components for biochemical and genetic study and that functional analysis of imprisoned cells is frequently inhibited by sampling luminal contents. Additionally, normal mechanical cues such fluid shear stress, tension, and compression that affect cell culture activity are typically not presented to cells. The inability to analyze how cultured cells interact with immunological and circulating blood cells due to the lack of fluid flow. Because of this, the introduction of microfluidics systems has led to the development of new, complicated systems to create cell cultures that are closest to simulating in-vivo conditions [15].

1.1.3 Dynamic cell culture

To overcome the drawbacks that cell culture systems based on static models had, microfluidic technology started to be applied to biological assays around three decades ago. In order to replicate the function of in-vivo cells, microfluidics technology was developed [16]. Microfluidics overcomes their limitations and makes it possible to examine a wide range of physiological phenomena by giving researchers control over a number of system parameters that are difficult to regulate with 3D static cultures (scaffolds or hydrogels) [16].

A novel type of cell culture vessel that enhances the ability to manage the local cellular microenvironment is the microfluidic system [17]. When culture systems are scaled down, some physical features alter, which is where microfluidic cell culture's advantages lies [18]. The cell microenvironment of cultivated cells is impacted by the physical layout of microfluidic devices. Walker et al. [19], who introduced the concept of effective cell culture volume as an indicator of cellular control over the microenvironment in the cell

culture device, described design considerations for practical application of microfluidic devices in cell biology.

Simple 2D cell cultures served as the foundation for the earliest dynamics systems. It has been demonstrated that these 2D perfused systems, as opposed to static cell culture methods, better preserve liver cell activities [20]. By managing the exchange of nutrients and the presentation of both chemical (like soluble stimuli) and physical (like mechanical, electrical) signals, the perfused culture system are intended to accurately control the in-vitro cell culture environments [21]. However, more realistic models using co-cultures arose as flat-plate culture systems, despite the fact that these 2D systems deliver false information in spite of better mimicking a genuine microenvironment than static systems. In these systems, the seeded cells may adequately touch the culture medium, allowing for appropriate mass transfer, and the cells can stick to the plates to form a homogeneous microenvironment. A system like this can also be easily scaled up, enabling high-throughput screening or research into zonation-dependent phenomena related to drug toxicity and metabolism [22]. The cells are subjected to high-shear stress in flat-plate culture techniques, which is a significant drawback. Consequently, they may detach from their scaffold and quickly lose their viability and function because they are anchor-dependent cells.

A lot of work has gone into developing systems to imitate the natural phenotypic of cells in 3D environments during the past few decades, thanks to advancements in microfabrication and microfluidics [23]. Organ-On-a-Chip (OOC) or microphysiological systems are the name given to these systems, which are ultimately intended to develop in vitro models of human organs [16].

In order to simulate the physiological activities of tissues and organs, OOC systems are microfluidic devices for cultivating living cells in continuously perfused chambers [24]. It provides excellent capabilities for developing miniature in vivo physiological models that replicate cell interactions and model body metabolism in both healthy and pathological conditions. These OOC systems can be built in various ways, but their most basic design centers on a single, perfused, microfluidic chamber that houses a single type of cultivated cell that performs the functions of a particular tissue or organ type. In more intricate designs, porous membranes that are bordered on opposite sides by various cell kinds are used to connect two or more microchannels in order to simulate the interfaces between various cell types. Physical forces, such as physiological levels of fluid shear stress, cyclic strain, and mechanical compression, can be incorporated into these systems [25].

A Human-On-a-Chip system has recently shown significant potential thanks to the integration of an increasing number of functioning organs on one platform [26]. When two or more organs are grown in linked microfluidic systems, the concept is also known as multi-organ-on-a-chip. Multiple organ fusion has already been attempted in the past [27][28][29]. One of the earliest studies published is Shuler et al.'s [30] platform, which has compartments for the liver, the lung, and other tissues. This work demonstrated the great potential of multi-organ integration. Due to numerous obstacles, such as the need to sustain a range

of cell types on a single device, employ a recirculating common medium, replicate inter-organ interactions, and transport nutrients and soluble substances at a physiologically relevant level, achieving this goal is not a simple process. This field of technology is still developing. The development of many organ chips will be necessary in order to create a Human-on-a-Chip system, which has great promise for the future of both industrial and medical applications, despite their challenges.

1.1.4 Open-well in vitro devices

In vitro devices of the open-well variety are frequently employed in cell culture investigations. These gadgets, as their name suggests, have a flat, open well where cells can develop in a liquid rich in nutrients.

The adaptability of open-well devices is one of their benefits. They are ideal for a wide range of applications, from fundamental cell biology to drug development and toxicity testing, and they may be employed with a variety of cell types. In addition, compared to conventional static cultures, open-well systems give researchers better control over the cellular microenvironment. For instance, gas-permeable membranes between the liquid and gas phases can assist in controlling oxygen levels, which is crucial for preserving cell viability and functionality [31]. However, open-well technology has some drawbacks. They might not precisely reflect the three-dimensional structure of tissues and organs in vivo, which can restrict their utility in some applications. This is one of their biggest drawbacks. It may also be challenging to replicate physiological flow rates and shear pressures, which is crucial for researching vascular and other fluid-responsive systems [2].

1.1.5 Closed-well in vitro devices

Closed-well in vitro devices are a type of cell culture system that features wells with lids or caps that seal off the cell culture from the external environment. These devices are commonly used in high-throughput screening applications, as well as studies involving infectious agents or hazardous materials.

The capacity of closed-well in vitro devices to shield samples from contamination by bacteria and airborne particles is one of its key benefits. When working with delicate cell types or biological materials that demand sterile settings, this is very crucial. In addition, the sealed construction permits the manipulation of gases and liquids inside the well, which can be crucial for specific kinds of research [32].

However, closed-well in vitro devices have some drawbacks. Due to the sealed construction, it may be challenging to regulate the cellular microenvironment, which can affect cell growth and function by determining the amount of oxygen and nutrients available. Additionally, the comparatively limited well volumes in these apparatuses may restrict their use for specific kinds of studies, including the mass generation of cells or tissues [2].

1.2 Main applications of MPSs

The physiological events that define the human immune response may be better understood with the help of in-vitro models of human cell culture [33]. OOC systems have developed into a potent technique to circumvent drug testing on animals as a result. Animals have been used successfully to improve understanding of human disease and have significantly contributed to the development of potent medicines for a long time [34]. They have also been employed frequently as models to investigate human reaction [35]. Such models, however, invariably show variations from human metabolism and illness states and may thus correlate poorly with human circumstances [36]. It becomes obvious that using human cells is necessary due to the important variations between human and animal metabolism. The community of those working in the life sciences is very interested in new methods that improve the biological relevance of cell culture models.

Traditional in-vitro models are typically composed of a single cell type, making them simple enough to be robust and suitable for high throughput research [37]. Unfortunately, this simplicity makes it difficult for them to accurately represent complex pathophysiology behavior that does not closely mimic in-vivo microenvironments. As opposed to 2D culture models, OOC models are biomimetic systems that depict the essential functional components of living human organs. In order to simulate a full body system, these microengineering co-culture models recreate the complex interactions between various cell types, from a single atom to a multi-organ system [80]. These systems may be employed as specialized in-vitro models that allow for intricate biological process simulation, mechanistic analysis, and pharmaceutical modification [38].

These cell-based assays have been a crucial component of the drug development process since they offer a quick, easy, and affordable approach to bypass extensive and expensive animal testing. When compared to animal models, these systems are more simple, making it possible to study cell signaling by continuously seeing the metabolites moved from one tissue to another. This enables the study of intricate physiological processes and, as a result, the comprehension of how metabolites affect a particular tissue's or organ's function as well as the manipulation of the healthy/diseased state.

1.3 Different types of MPSs

The following lists some of the most important articles on cell culture of various organ types with the common aim of examining their function or/and reaction to various medications.

1.3.1 Lung-on-a-chip

The crucial functional alveolar-capillarity interface of the human lung was recreated by the first biomimetic microsystem, according to Huh et al. [39] (Figure 3). The responses to bacteria and inflammatory cytokines introduced in the alveolar space are replicated by their device at the level of complicated integrated organ responses. The microsystem combines controlled vacuum with microfluidics to create cyclic stretching, which improves epithelial and endothelial uptake of nanoparticles and stimulates their transport into the microvascular channel.

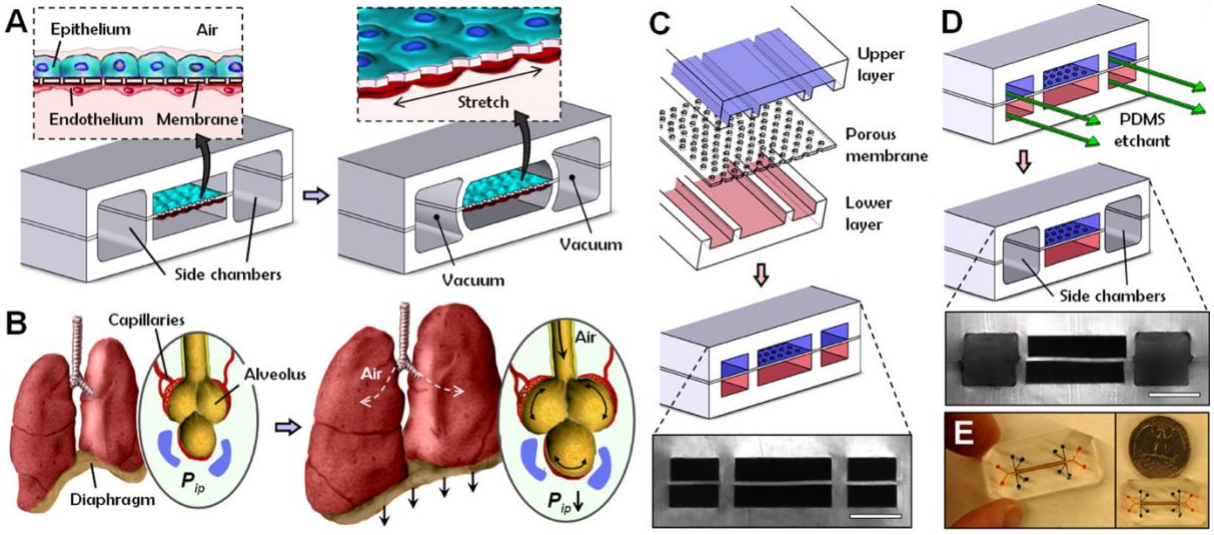

Figure 3. *Biologically design of a human breathing Lung-On-a-Chip microdevice. (A) The alveolar-capillary barrier is created by the microfabricated lung mimic device using compartmentalized PDMS microchannels.(B) The act of breathing through a living lung. (C) Microchannels made of PDMS with a series of holes that have an effective diameter of 10 m. (D) Mechanical stretching is achieved by applying vacuum to two sizable side chambers. (E) Photographs of the microfluidic apparatus* [39].

1.3.2 Gut-on-a-chip

Kim et al. [40] proposed a biomimetic Gut-On-a-Chip microdevice that mimics the intricate structure and physiology of a real gut and is made up of two microfluidic channels separated by a porous flexible membrane coated with ECM and lined with human intestinal epithelial cells (Figure 4). In a controlled microfluidic environment that is suitable for transport, absorption, and toxicity studies, this microdevice recapitulated numerous dynamic physical and functional characteristics of the human intestine that are essential for its function. As a result, it should be extremely useful for drug testing as well as the creation of new intestinal disease models.

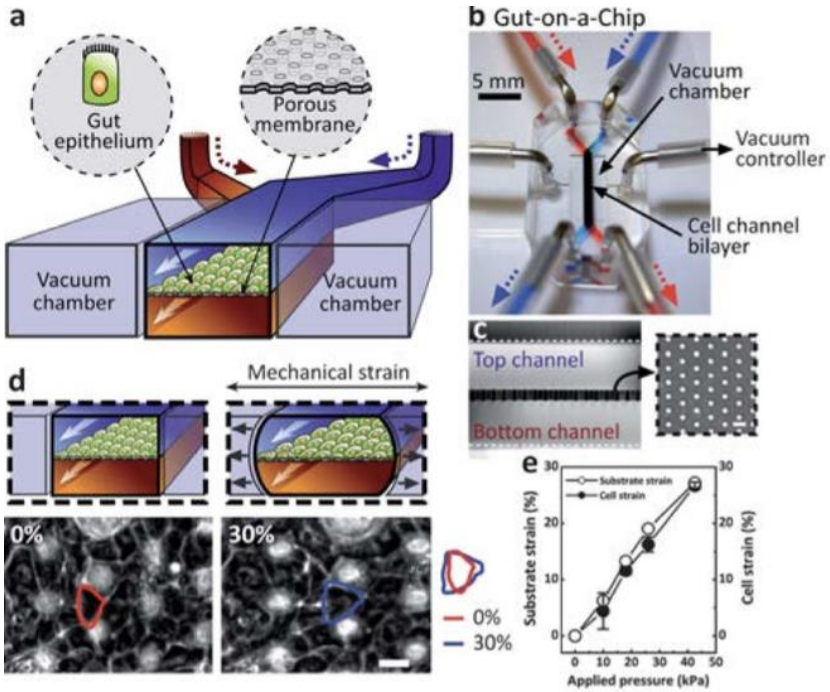

Figure 4. *a) A schematic of the Gut-On-a-Chip device showing the flexible porous membrane, (b) a photographic image of the device composed of clear PDMS elastomer, (c) a cross-sectional view of the top and bottom channels (both 150 µm high), (d)schematics (top) and phase contrast images (bottom) of intestinal monolayers cultured within the Gut-On-a-Chip in the absence (left) or presence (right) of mechanical strain, (e) quantization of the mechanical strain produced in the PDMS membrane (open circles) and in the adherent gut epithelial cells (closed circles) as a function of pressure applied by the vacuum controller* [40].

1.3.3 Liver-on-a-chip

The creation of a Liver-On-a-Chip platform for long-term cell culture of 3D human spheroids for drug toxicity assessment was described by Bhise et al. [41] (Figure 5). A bioprinter might be connected to the designed bioreactor to create 3D liver structures made of spheroids enclosed in photocrosslinkable gelatin methacryloyl hydrogel. Furthermore, by allowing direct access to the hepatic construct during the experiment without endangering the platform's operation, the bioreactor's design enabled in-situ monitoring of the culture environment.

1.3.4 Kidney-on-a-chip

Jang et al. created one of the first Kidney-On-a-Chip systems [42]. Using a collection Duc-On-a-Chip (Figure 6), they investigated a renal tubular epithelial cell culture exposed to luminal shear stress and a transepithelial osmotic gradient. They tested a wide range of metrics to show the effects of alterations in the luminal or basolateral microenvironments with the intention of creating an easy-to-use tool for drug screening to examine renal physiological and pathophysiological characteristics.

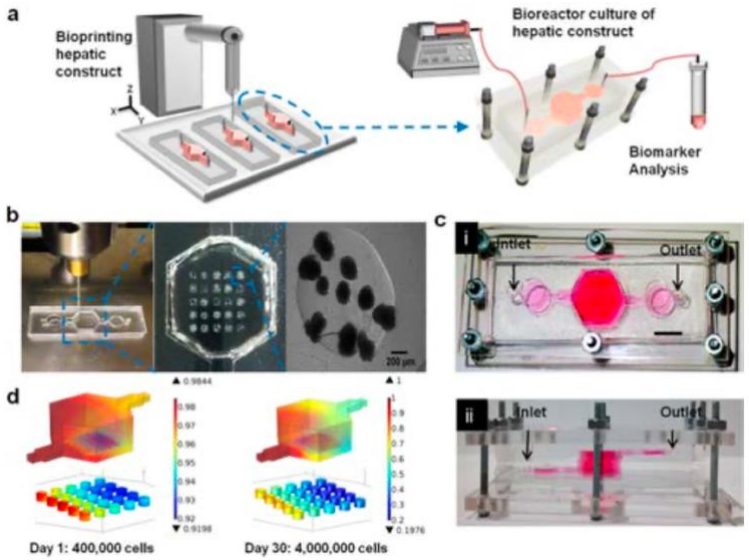

Figure 5. *(A) A schematic of the hepatic bioreactor culture platform with a bioprinter and a biomarker analysis module is shown. (B) A photocrosslinkable hydrogel-based hepatic construct is bioprinted inside the bioreactor as a dot array. (C) Top and side views of the assembled bioreactor with the inlet and outlet fluidic ports are shown. (D) The bioreactor has an oxygen concentration gradient* [41].

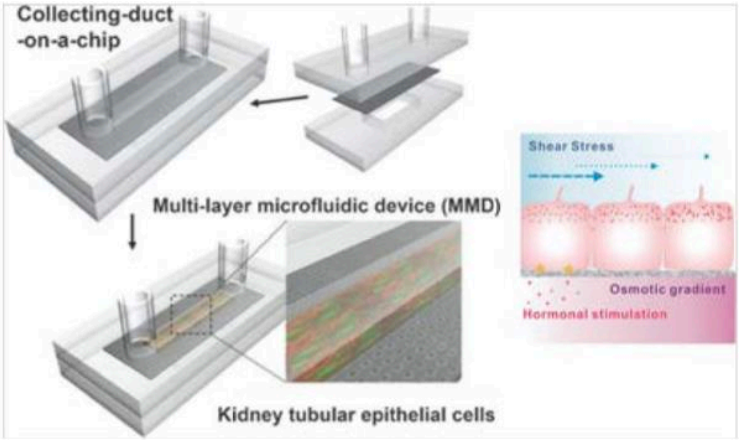

Figure 6. *Schematic of the Duct-On-a-Chip, which was used to explore how changing environmental conditions affected the culture of kidney cells inside the channel* [42].

The key studies on microphysiological models that have been created in recent years to simulate organ and tissue, as well as their application, are summarized in Table 1. Even though many significant microenvironmental components that can be modified artificially in vitro have been found, much work still has to be done. Specifically, how to create simple, high-throughput, and repeatable in-vitro systems that produce these microenvironmental elements. As the initial conditions are slightly altered, the great complexity of these models becomes more unpredictable and subject to changes. Less reliable measurements are produced as a result of the model's unpredictability. Because of this, the most popular in-vitro models continue to be based on straightforward conventional assays.

Table 1. *Summary of microengineered Organ-On-a-Chip models and their main applications.*

	Cell types	**Applications**	**References**
Lung-on-a-chip	Lung epithelial cells, endothelial cells	Study of lung diseases, testing of drug toxicity/effects	[39][43]
Hear-on-a-chip	Cardiac cells, endothelial cells	Study of cardiac diseases, drug screening	[43]
Liver-on-a-chip	Hepatocytes, endothelial cells, stellate cells	Study of liver diseases, testing of drug metabolism/toxicity/effects	[43][44]
Kidney-on-a-chip	Renal epithelial cells, endothelial cells	Study of kidney diseases, testing of drug toxicity/effects	[43]
Gut-on-a-chip	Intestinal epithelial cells, endothelial cells, immune cells	Study of gut diseases, testing of drug toxicity/effects	[43][40]
Blood-brain-barrier (BBB) OOC	Brain endothelial cells, astrocytes, pericytes	Study of brain diseases, drug delivery to the brain	[43][45]

2.

Analysis of the main validation and characterisation assays

2.1 Introduction

Standardization of MPSs implies robustness and reproducibility of these devices and is desirable from an industrialization/production and regularization point of view. However, due to the early stage of academic and commercial development of this technology, no standardization procedure exists in the literature to date. Therefore, experts recommend focusing on the characterization or qualification of these devices [46]. This characterization and qualification of the MPS involves testing the various elements that make up the device to ensure that its configuration mimics the physiology of the human structures represented, behaving and offering values as similar as possible to those of in vivo tissues.

Specifically, barrier dysfunction is most often characterized by two main parameters: macromolecular permeability, which is a direct indicator of solute flow across the cell barrier, and electrical resistance, which reports the tightness of the cell monolayer to ion flow. Macromolecular permeability is determined with a fluorescently labeled macromolecular tracer, including dextran and polystyrene microspheres. On the other hand, transepithelial electrical resistance (TEER) is a measure of ionic permeability across intercellular clefts. Thus, TEER and FITC-dextran flux are two distinct measures of permeability that characterize different parameters, which can be regulated independently [47].

In this chapter, the main validation tests for different parameters of microphysiological systems will be analyzed and examples will be presented.

2.2 Microfluid characterization

2.2.1 Micro Particle Image Velocimetry (Micro-PIV)

Micro Particle Image Velocimetry (micro-PIV) is widely used in organ-on-a-chip systems to quantitatively analyze the fluid flow. This technique employs microscopic particles, such as fluorescent beads, to trace the flow of fluids in microfluidic devices. The flow information is obtained by measuring the particle displacement using high-speed cameras. The advantages of micro-PIV include its ability to precisely measure flow dynamics, visualize fluid patterns, and simulate the behavior of organ systems for better understanding of disease progression [48].

In organ-on-a-chip studies, micro-PIV has been used to measure blood flow in a T-shaped microfluidic channel that simulates the blood flow in the liver. The technique has also been employed in studies examining the metastatic development of breast cancer [49]. In this system, micro-PIV is used to measure the fluid flow in a bone-mimicking matrix integrated with a microfluidic chip. Micro-PIV measurements have also been applied to multi-organ chips, where the flow dynamics of blood between the heart, liver, spleen, and lungs were quantified [50].

2.2.1.1 Case study

Micro-PIV has been used in a case study to describe the flow of a four-organ chip that simulates the intestine, liver, kidney, and skin.

First, the mean flow rate is computed at locations (Figure 7.b) A, B, and C for the substitutive blood circuit and at points D and E for the excretory circuit. Then, phosphate-buffered saline (PBS) is used to resuspend human red blood cells (RBCs), and a dilution of 5x108 cells per mL is created. The liver compartment is filled with 600 µL of RBC solution, while the excretory circuit's reservoir is filled with 300 µL. PBS is used to fill the chip's remaining space. In order to monitor the movement of the RBCs, a high-speed CMOS camera is connected to an inverted microscope. The exposure time is set to 4 µs per single image; the frame rate varies, depending on the size of the region of interest (up to 2732 fps) [51].

The displacement of the RBCs, the maximum velocity of the laminar plug flow in the channels, and the flow rate at each site may all be measured using this assay.

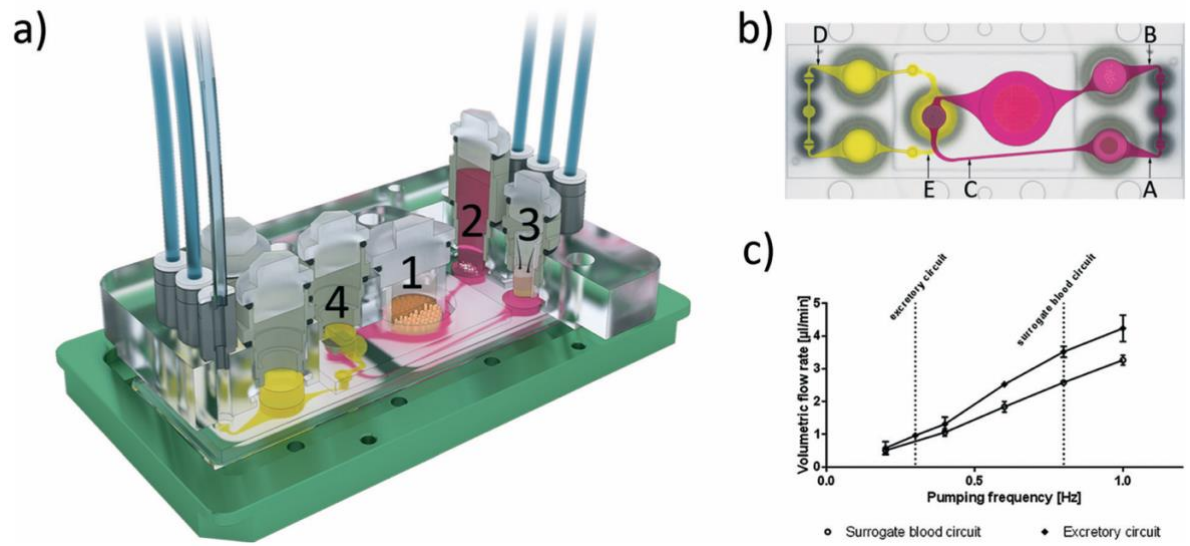

Figure 7. *a) 3D view of the device showing two polycarbonate cover plates and a PDMS-glass chip with a substitute blood flow circuit (pink) and an excretory flow circuit (yellow) (footprint: 76 mm x 25 mm; height: 3 mm). The four tissue culture compartments for intestine (1), liver (2), skin (3), and kidney (4) tissue are represented by numbers. Each tissue culture compartment's central cross-section is shown, aligned with the connecting microchannel. b) Using PIV, evaluating fluid dynamics in the 4OOC. Three measurement spots (A, B, and C) in the surrogate blood circuit and two spots (D, E) in the excretory circuit are shown in the top view of the four-organ chip arrangement. c) The average volumetric flow rate of the excretory and surrogate blood flow circuits displayed against the frequency of pumping. The vertical lines represent co-culture trials that were conducted at 0.8 Hz and 0.3 Hz, respectively. Standard error of the mean appears as error bars. [51]*

2.3 Permeability assays

2.3.1 Transepithelial electrical resistance (TEER)

Trans Epithelial Electrical Resistance (TEER) is a widely used impedance-based monitoring technique that provides label-free, quantitative, and real-time measurements that correlate to the formation of tight junctions between the cells in the cell barrier (2D or 3D), thereby giving a good indication of barrier integrity. TEER is especially useful for barrier tissue models. Before they are assessed for the ability to transport medications or substances, cellular barriers' integrity is strongly indicated by TEER values [52].

Together, epithelial and endothelial cells close the intercellular space in living organisms, forming a physical barrier that divides the apical and basolateral compartments. Due to the structure of the tissue, two pathways exist for solutes to pass over the barrier: the transcellular pathway, which involves passing through the cell membrane, and the paracellular method, which involves moving between cells. It takes a driving force, such as a concentration gradient, an electrical potential difference, a hydrostatic pressure, or an osmotic gradient, to move ions and molecules through the paracellular route because it is passive [53]. The majority of ion passive transport is accounted for by an electrochemical gradient, which is created when chemical gradient and electric voltage are combined. Moreover, cells feature an active transcellular transport mechanism that uses transmembrane proteins to move molecules against their electrochemical gradient. In the transcellular route, this active transport coexists with the passive one [54][55].

The paracellular resistance and the transcellular resistance, the total of two ion conductive routes, are determined by TEER measurements. A typical TEER measurement process entails:

1. Measuring the blank resistance (device without cells) R_{BLANK};
2. Measuring the total resistance (device with cells) R_{TOT};
3. Subtracting the total resistance and the blank resistance.

$$R_{TISSUE}\ (\Omega) = R_{TOT} - R_{BLANK} \quad (1)$$

4. Normalizing the obtained net value on the culture area A_{TISSUE} (cm^2).

$$TEER\ (\Omega \cdot cm^2) = R_{TISSUE} \cdot A_{TISSUE} \quad (2)$$

Typically, the final TEER value is provided in (Ohm x cm^2). Table 2 lists typical TEER values for the intestinal barrier.

2.3.2 Electrochemical permeability assay

These assays involve measuring the transport of ions across a cell monolayer in response to an electric field. The magnitude of the electric current that flows across the cell layer is indicative of its permeability and the barrier function of the cell monolayer.

Electrochemical permeability assays have been used in various organ-on-a-chip models, such as the liver and intestinal mucosa. For example, in a study on a liver-on-a-chip model, an electrochemical microfluidic permeability assay was developed to assess the barrier function of endothelial and epithelial liver cell monolayers [56]. The assay enabled the rapid and quantitative measurement of barrier integrity and permeability [57], which is important in evaluating drug toxicity and predicting drug metabolism in the liver.

Table 2. Typical TEER values for the intestinal barrier.

Cell types	TEER (Ohm x cm^2)	References
Gastric mucosa (in vivo)	2000	[58]
Colo (in vivo)	300-400	[58]
Small intestine (in vivo)	50-100	[58]
Caco-2	1100-1350	[59]

2.3.3 Fluorescent tracer-based permeability assay

In these tests, a fluorescent tracer is placed on one side of the cell layer in the microfluidic device, and the amount of the tracer that passes to the other side is tracked over time. The strength of the fluorescence signal reveals the cell monolayer's permeability and barrier capabilities.

The lungs, liver, and intestine are just a few of the organs-on-a-chip models that have utilized fluorescent tracer-based permeability experiments. For instance, a fluorescent tracer-based permeability experiment was employed to show how the endothelial cell monolayer acts as a barrier in response to inflammatory stimuli in a study using a lung-on-a-chip model [60]. Similar to this, a liver-on-a-chip model used fluorescent tracer-based tests to look at the hepatocyte layer's function as a barrier and gauge how the liver reacts to toxins and drugs [56].

2.4 Oxygen transport characterization

2.4.1 In-line fluorescent sensing

In-line fluorescent sensing assays are a promising tool for monitoring oxygen transport in microphysiological systems (MPS). These assays use fluorescent probes that can detect and measure changes in oxygen concentration in real-time, allowing researchers to assess the oxygenation status of MPS and optimize their performance.

Several studies have utilized in-line fluorescent sensing assays for oxygen transport characterization in MPS. For example, a study published in Scientific Reports developed an in-line fluorescent sensor to monitor oxygen delivery in a liver-on-chip system [61]. Similarly, another study published utilized a hypoxia-responsive fluorescent probe to monitor oxygen gradients in a tumor-on-a-chip model [62].

One case worth mentioning for its success is the study of the effect of interleukin-2 (IL-2) for the treatment of pulmonary edema. The assay used to characterize oxygen transport in the air channel (Figure 8) the In-line fluorescent sensing technique. 4 days before performing the assay, they administered IL-2 to induce fluid leakage into the air channel. The supplemented medium was deoxygenated by bubbling 95% N_2 and 5% CO_2 and FITC-inulin was infused into the device. The flow was regulated to 500 µL/h using a syringe pump, and O_2 concentrations were measured using the sensor placed above and below the air and capillary microchannels [63].

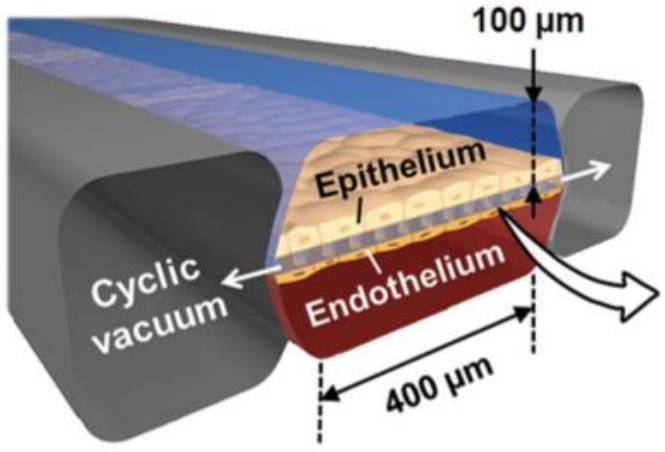

Figure 8. *In a microengineered lung-on-a-chip that replicates the lung micro-architecture and breathing-induced cyclic mechanical distortion of the alveolar-capillary interface, IL-2-induced pulmonary edema is mimicked. Alveolar channel is the top "air" portion, and vascular channel is the bottom "liquid" portion. A top-down view of the apical surface of the alveolar epithelium, which is kept at an air-liquid interface in the upper microchannel, is shown in the phase-contrast image [63]. 200 mm scale bar.*

3.

Protocol for monitoring membrane permeability in real time

3.1 Introduction

After studying the diverse devices that are currently at an early stage of academic and commercial development, and evaluating the various tests used to characterize the passage of molecules of different molecular weights through human body membranes mimicked in MPSs, a non-invasive protocol is developed to monitor membrane permeability in real time. The assay involves the use of confocal microscopy to obtain direct images of the in situ evolution of fluorescence in the trench of the membrane chip directly below the barrier after the addition of a fluorescence tracer.

Confocal microscopy allows observation of thick sections of specimens with high optical resolution, facilitates the reconstruction of three-dimensional images and enables in vivo imaging without the artifacts induced by tissue processing [65]. However, it is expensive compared to conventional microscopy, so fluorescence microscopy can be used as an alternative. This second, less expensive, analytical technique is also commonly used and, despite having lower optical resolution, ultimately also allows detection of the presence and localization of fluorescent molecules in the sample.

Two fluorescence tracers of different molecular weight are selected to perform the tests described below. Fluorescein isothiocyanate-dextran (FITC-dextran) 10 kDa will be used for macromolecule permeability assays while FITC-dextran 5 kDa will be used for measurements on a smaller scale. These markers have been chosen because they are some of those commonly used regardless of the barrier tissue to be characterized. However, among the different molecular weights in which dextran is commercially available, depending on the MPS, other MWs of FITC-dextran can be considered.

Although FITC-dextran is sensitive to pH and there are conjugations with greater photostability such as Alexa Fluor (AFA568) [66], the former turns out to be more economical. Dextran is a polymer, composed of glucose molecules linked together by mostly α-D glycosidic bonds. Dextran with a lower MW show slightly less branching and a narrower MW distribution range, while those with a MW above 10kDa behave as if they are highly branched. As MW increases, dextran molecules achieve greater symmetry [67]. Its excitation is best at a wavelength of 493 nm and fluorescence is measured at 518 nm. As mentioned, their fluorescein moiety charge state depends on the pH and ionic strength of the medium, in turn modifying the fluorescence intensity. The maximum value of the fluorescence intensity is observed at pH > 8 [68].

3.2 Cell-free diffusion assay

Before measuring cell permeability it is necessary to validate the device in situ with analytical and experimental studies of free diffusion described by Fick's law, since this rule has proved to be a good description of the physics governing the evolution of fluorescence in these experiments [69].

The cell-free experiment is initiated by washing the device with fresh media of phosphate buffered saline (PBS). The membrane is then coated with a mixture of collagen/fibronectin, which is necessary to slow diffusion through the highly porous membranes. The entire volume of the upper channel is then replaced with a solution containing a concentration of 2 mg/mL of FITC dextran (10 kDa) re-suspended in sterile PBS or saline. The concentration of FITC-dextran can be adjusted depending on the imaging application, using higher or lower concentrations of FITC-dextran, ranging from approximately 0.1 to 10 mg/mL [70]. The confocal microscope image plane is focused below the membrane in the lower channel and the imaging sequence is initiated within 3 seconds of adding the tracer, which diffuses from the upper to the lower channel. Images are taken every minute for a total time of 10 minutes. Finally, the data are fit to equation (1), a free diffusion equation derived from Fick's law equated to the complementary error function, *erfc*, to solve for the diffusion coefficient, *D*, and the fluorescence intensity at infinite time, F_∞:

$$\frac{F_{x,t}-F_b}{F_\infty-F_b} = erfc\left(\frac{x}{2\sqrt{Dt}}\right) \quad (3)$$

where $F_{x,t}$ is the fluorescence intensity at position *x* and time *t*, F_b is the background fluorescence intensity measured at time t=0, *t* is the diffusion time, and *x* is the diffusion distance.

The procedure is repeated in a new culture for the 5 kDa dextran molecule with the same concentration.

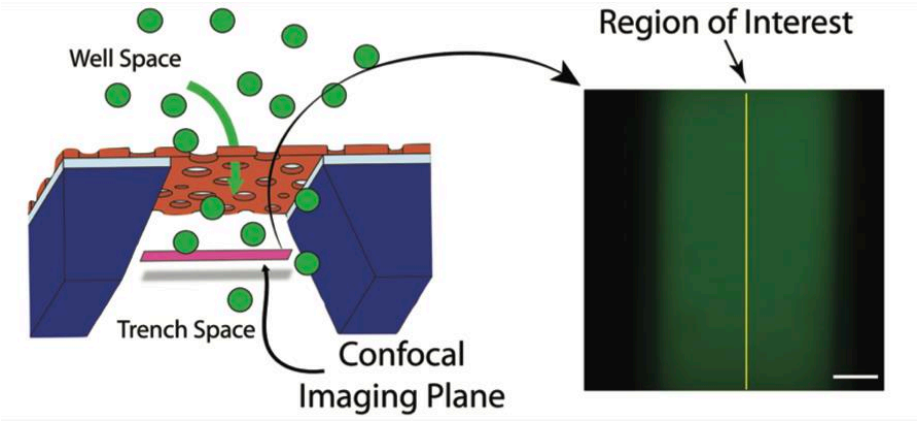

Figure 9. *On cell-free devices, optimize in situ permeability assays. A confocal microscope's imaging plane is focused inside the chip's trench, beneath the membrane. From the well into the trench, dye diffuses (left). An example of a corresponding image is shown on the right, where a yellow line indicates a linear region of interest in the membrane's middle where 1D diffusion accurately depicts the progression of fluorescence [50].*

3.2.1 Expected results

***Table 3**. Dextran diffusion coefficients according to the type of membrane.*

Barrier	Molecular Weight Range (kDa)	Diffusion coefficient (cm^2/s)	References
Biological Membranes	3.2 - 70	$4.5e^{-08}$ to $2.6e^{-10}$	[71]
Synthetic Membranes	4 - 500	$5.5e^{-08}$ to $4.6e^{-11}$	[72]
Hydrogels	4 - 200	$1.2e^{-07}$ to $3.9e^{-10}$	[73]

3.3 Cell culture permeability assays

After 2 to 5 days of cell culture and perfusion, we can proceed to characterize the barrier function that restricts the passage of foreign materials in static and dynamic cultures with monoculture or co-culture of cells.

3.3.1 Static conditions

Once the cells have been seeded on the membrane, the culture is maintained under static conditions for 24 h before continuing with the assay. After this time, the device is washed with free media and a concentration of 500 μg/mL consisting of FITC-dextran 10 kDa and phosphate buffered saline (PBS) is added to the top channel of the chip. The distribution of the fluorescence marker is then recorded with the confocal microscope for 48 hours for a total of six time points (0h, 3h, 6h, 9h, 12h, 24h and 48h) in the upper and lower channels simultaneously. Finally, the data are fitted to equation (2), a steady flow equation, to solve for the barrier permeability, P. This equation is derived from the equation for molecular transport into a semi-infinite space from a boundary undergoing steady flow:

$$\frac{F_{x,t}-F_b}{F_0-F_b} = \left(2\sqrt{\frac{t}{\pi D}}e^{\left(-\frac{x^2}{4Dt}\right)} - \frac{x}{D}erfc\left(\frac{x}{2\sqrt{Dt}}\right)\right)P \quad (4)$$

where $F_{x,t}$ is the fluorescence intensity at position x at time t, F_b is back-ground fluorescence intensity, F_0 is source fluorescence intensity, t is time of diffusion, D is the diffusion coefficient of the molecule, and x is the distance of diffusion.

The procedure is repeated in a new culture for the 5 kDa dextran molecule.

3.3.2 Dynamic conditions

After the cell culture and perfusion days have elapsed, the culture is maintained for 2 days at a growth medium flow rate that will depend on the barrier tissue as shown in Table 2. Afterwards, the same procedure described in the previous section is implemented.

Table 4. *Flow rate values induced during cell culture as a function of culture cell composition.*

Cell type	Flow rate	References
Gut	5 μL/min	[74]
BBB	1.5 μL/min	[75]
Lung	0.2 μL/min	[76]
Liver	3.3 μL/min	[77]

3.3.3 Expected results

Table 5*. Dextran diffusion coefficients according to membrane cell composition.*

Tissue barrier	Molecular Weight Range (kDa)	Diffusion coefficient (cm^2/s)	References
Blood-Brain barrier	3 - 70	$1.7e^{-07}$ - $1.2e^{-10}$	[78]
Small intestine	4 - 150	$6.8e^{-07}$ - $8.8e^{-09}$	[79]
Endothelial cells	4 - 20	$2.8e^{-06}$ - $1.6e^{-08}$	[80]
Intestinal Epithelium	4 - 70	$1.1e^{-07}$ - $2.1e^{-12}$	[81]

3.4 Execution of the protocol

In this last section of the main axis of the thesis, the protocol defined above is executed to verify its feasibility regarding the characterization of OOC devices. The assay is applied to a physiological system that mimics the exocrine pancreas. As this device has a cell culture and is not equipped with any perfusion pump, a cell culture membrane permeability test is performed under static conditions.

The device has been loaned by doctoral students at the Politecnico di Milano.

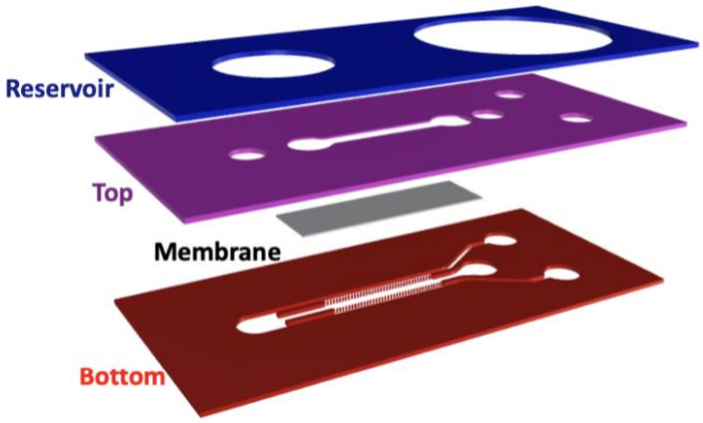

Figure 10. *Device schematic diagram.*

The following steps have been taken with seeded cells in order to perform fluorescence imaging:

- For each device, cells were treated with paraformaldehyde for 40 minutes, which ensures cell fixation and a good conservation of cellular structures due to its capacity to bind free amino groups of amino acidic lateral chains;
- TritonX100 was used to permeabilize the cell membrane in order to make phalloidin access easier. Because it is a mild agent and non-denaturant, it is frequently used to solubilize proteins. It is also utilized to permeabilize live cell membranes. For 10 minutes, cells were exposed to a TritonX100 solution in PBS (0.5%).
- Bovine serum solution (BSA), a blocking buffer that saturates the excess protein binding sites, has been used to block cellular proteins [60]. For the 1-hour cell treatment, BSA has been diluted in PBS to provide a 1% BSA solution.
- Phalloidin (1:60 μL in BSA; ThermoFisher Scientific) was used to incubate cells for 40 min. Phalloidin is a fluorescent dye that is primarily used to mark and detect F-actin in cell cultures.
- Next, cells were treated for 5 minutes with a mixture of PBS and the blue fluorescent dye 4',6-diamidino-2-phenylindole (DAPI), which binds to the AT sections of DNA. In fluorescence microscopy, it serves as a nuclear stain. The final solution was created by dilution of an

intermediate solution, which was created by mixing 2 μL of DAPI with 100 μL of PBS, into 1 mL of PBS.

Fluorescence data collected using a plate reader is presented in table 6. The samples with Dextran put into the top layer's channel are designated by the letter 't' while the samples with Dextran introduced into the central channel of the bottom layer are designated by the letter 'b'. The % values for 's' refer to the amount of dextran that has passed the porous membrane, i.e. the amount of dextran in the lower channel; and vice versa.

Table 6. *Fluorescence data obtain with plate reader.*

	number of dextran molecules					
Time (minutes)	**t1**	**t2**	**t3**	**b1**	**b2**	**b3**
0	536	536	536	536	536	536
30	117	231	95	46	125	21
60	22	4	59	51	26	8
90	12	24	7	19	9	14
120	4	4	3	1	4	31
150	4	4	8	14	4	2

Table 7 presents the results of an Excel statistical analysis of the fluorescence data. The average for each time step and condition, along with the corresponding standard deviation, were determined using the samples' fluorescence measurements;

The results of an Excel-based statistical study are graphically displayed below (Figure). Samples with Dextran inserted into the top layer's central channel are referred to as "Top" samples, while samples with Dextran introduced into the bottom layer's central channel are referred to as "Bottom" samples.

According to the first two blue columns (0 and 30), the majority of the Dextran crosses the membrane in the first thirty minutes. Because the blue columns' data trend is the same as that of the first two orange columns, this rapid passage of dextran molecules occurs in both directions (from up to down and from down to up). Dextran molecule flow then gradually declines until it is nearly nonexistent (less than 10%) in the final two columns for both the blue and orange ones. The data has a significant standard deviation, as seen by the graph. These data imply that the membrane's porosity causes permeability to occur quickly, during the first several minutes. This is crucial in order to mimic cell contact between two compartments,

which occurs in the PDAC microenvironment where pancreatic cancer cells produce certain substances that promote PSC proliferation [82].

Table 7. *Fluorescence data statistical analysis.*

	%									
Time (minutes)	**t1**	**t2**	**t3**	**Average**	**Std. dev**	**b1**	**b2**	**b3**	**Average**	**Std. dev**
0	100	100	100	100	0	100	100	100	100	0
30	21,8	43,1	17,7	28	0,14	8,6	23,3	3,9	12	0,1
60	4,10	0,75	11,01	5	0,05	9,5	4,85	1,49	5	0,04
90	2,24	4,48	1,31	3	0,02	3,5	1,68	2,61	3	0,01
120	0,75	0,75	0,56	1	0,00	0,19	0,75	5,79	2	0,03
150	0,75	0,75	1,49	1	0,00	2,61	0,75	0,37	1	0,01

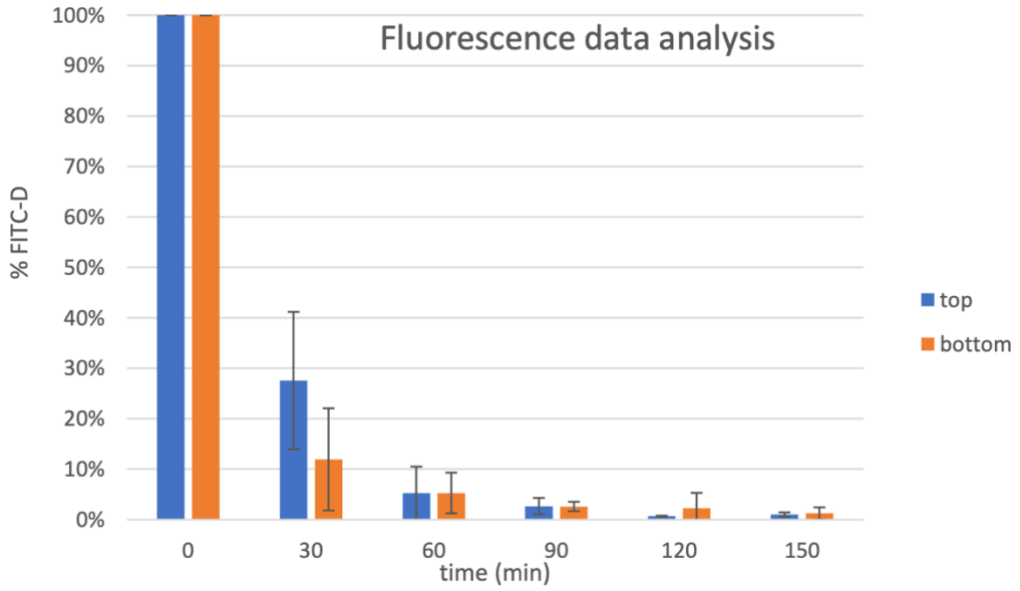

Figure 11. *Bar graph of average values, with the related standard deviation values.*

Environmental study

Because this final year work was entirely computer-based, the environmental impact depends on several factors, such as the energy efficiency of the computer, the energy source used to power it, and the disposal process of the equipment at the end of its useful life.

In addition, the use of computers and other electronic equipment can also generate electronic waste, which is often not properly managed and can have a negative environmental impact through the release of toxic substances.

It is important to mention that the environmental impact could have been mitigated if sustainable practices had been adopted in the research work, such as the use of renewable energy and, in the future, the responsible disposal of electronic waste.

Taking into account that a MacBook Air (2022) Apple M2 Chip has been used for about 600 hours with a maximum power consumption of 50 W/h, approximately 0.03 kg of carbon per hour will be produced, so that in total about 18 kg of carbon will have been produced to carry out this project.

Conclusions

Microphysiological systems (MPS) are powerful tools that can be used to model complex physiological processes in vitro. MPS can provide a more accurate representation of human biology than traditional cell culture models, and can be used to test the safety and efficacy of drugs and other therapeutics prior to clinical trials. MPS can also be used to study disease mechanisms and to develop personalized medicine approaches by using cells from individual patients. Furthermore, represent a major evolutionary step towards the eradication of clinical trials on animals. However, there are still challenges associated with the development and implementation of MPS, including the need to optimize culture conditions and to validate the systems to ensure that they are reproducing physiological responses accurately. Despite these challenges, the potential benefits of MPS for drug discovery and other applications make them a promising area of research and development for the future.

To overcome these challenges, it is essential to collaborate with domain experts, ensure transparency in the development process, adopt best practices in data management, and establish clear validation criteria that align with system requirements. Additionally, leveraging existing frameworks and tools for developing and testing MPS can significantly reduce development time and costs while improving system reliability and performance. Overall, overcoming these challenges requires a multidisciplinary and collaborative approach that combines expertise in engineering, life sciences, and data science. The combination of these approaches can help to advance the field of microphysiological systems and improve their use and impact in precision medicine and drug development.

At present, due to the early stage of development of these devices, it is very difficult to develop a universal protocol to qualify OOC systems. This has been pproven by implementing the membrane permeability assay with cell culture and under static conditions in the microphysiological system that mimics the exocrine pancreas. During the execution, steps not contemplated in the protocol had to be added and modified due to the configuration of the device. This leads us to think that, for the future, it would be more interesting if organizations such as the European Commission (EC) or the Food and Drug Administration (FDA) would first define a regulation that would reduce dissimilarities between devices and, for practical purposes and independently of the manufacturer, OOC systems would be the same from an ingestion point of view. It would then be possible to start defining a protocol to characterize them and, later on, to validate and standardize them.

Reference

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