organoids

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Review

Why Can Organoids Improve Current Organ-on-Chip Platforms?

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Abstract: Preclinical studies are the first stage of introducing a new potential drug to the pharmaceutical market. Many of the compounds with promising results approved in the preclinical stage show poor prognosis during the first stage of clinical studies, which is connected with inadequate in vitro and in vivo models used in this stage. Both basic in vitro models, and in vivo animal models do not represent the human conditions. Therefore, scientists work on creating an appropriate model that will highly reproduce the characteristics of the human body. The solution could be an organoids model: a laboratory-produced human miniature organ, grown in a specially designed Organ-on-Chip microfluidic tools. This review focuses on characterizing the 3D cell culture types, focusing mainly on organoids, the Organ-on-Chip approach, and presenting the latest reports about the application of their combination in biological research, including toxicological studies.

Keywords: organoids; Organ-on-Chip; toxicology; drug screening; 3D cell culture

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1. Introduction

The new drugs development is a long (~15 years) and costly process. The average cost of each new drug introduced to the pharmaceutical market is over 1 billion USD. For any pharmaceutical company, it is a great achievement for a drug candidate to go through the rigorous stage of preclinical research, in which data about the effectiveness and safety of a drug are collected [1,2]. Usually, the preclinical studies are carried out on in vitro cell culture models and in vivo animal models [3]. The main purpose of preclinical studies is to determine an initial, safe dose for the first treatment in humans and to evaluate the potential toxicity product. The failure rate in drug development is greater than 90% [4]. Four possible reasons for this score, namely, a lack of clinical efficacy (~50%), unmanageable toxicity (~30%), poor drug-like properties (~10%), and a lack of commercial needs and poor strategic planning (~10%), were identified [5]. The first two reasons relate to errors resulting from inadequate test models of the preclinical stage. In vitro models suffer from deficiencies in reproducing the characteristics of living organs that are critical to their function [6]. In contrast, animal models are limited by the phylogenetic discrepancy between laboratory animals and humans, resulting in poor predictions of drug responses. Moreover, high costs, time, as well as ethical concerns limit the use of animal models as tools for pharmaceutical research [4,6].

Because cells in 2D cultures were found not to behave the same as in vivo, 3D cultures became promising model systems for new drug developments [7]. Recent technological breakthroughs enable the creation of 3D culture models that are physiologically more relevant to the in vivo environment than a standard monolayer, such as spheroid or hydrogel cultures, or more advanced organoids [8]. Organoids are 3D cell structures that contain many types of cells, with a tissue structure and cell–cell interaction resembling many of the important features of an organ in vivo [9,10]. They are obtained from one or more stem cells that can self-organize into three-dimensional cultures [11]. Organoids are becoming

more complex as scientists explore the mechanisms involved in stem cell lineage and differentiation [12]. Organoids of many organs have already been developed, including the brain [13], liver [14], kidney [15], breast [16], and pancreas [17], or disease models [18,19]. They can be used in toxicity studies as well as for testing new pharmaceutical molecules and in personalized and regenerative medicine. However, researchers in the fields of physical, biological, and engineering sciences are trying to improve the conditions of 3D cultures, including organoids, to be able to produce and grow mini organs under laboratory conditions. Organ-on-Chip is an approach using microfluidic devices lined with living cells grown in fluid flow that can faithfully reproduce physiology and pathophysiology at the organ level [20]. The advantages of microfluidic cell culture systems include the presence of dynamic flow conditions and mechanical stimulations in the microchannels, or the reproducing of gradients and chemical concentrations with high sensitivity and precision. Moreover, compared to other available in vitro models, Organ-on-Chip systems strive to maintain a balance between high scalability and high physiological relevance [21,22].

In this review, after the description and characterization of preclinical models, with a particular focus on organoids, we move on to the description of the Organ-on-Chip approach. In the next step of the review, we will focus on describing specific examples of the use of organoids in the Organ-on-Chip approach. Finally, we will focus on the description of the future perspectives of combining the two innovative approaches, which are organoids and OoC.

2. Preclinical Models

Two-dimensional cell cultures (2D) are a conventional approach known since the 20th century and the most widely used by most researchers. In such a model, adherent cells are cultured as monolayers on a flat glass or polymeric surface [7]. Since 2D cultures are well grounded and tested, as well as cheap and easy to maintain, these systems form the basis of almost all current routine tests. However, this approach has many limitations, mainly because 2D cultures do not represent a real cellular environment—in the body, cells are not a monolayer structure but a complex, multicellular model surrounded by an extracellular matrix (ECM). The main limitation of the 2D approach is that it does not fully reflect the cell–cell and cell–extracellular environment interactions [23]. Moreover, there are many differences between cells in the body and cells cultured in a monolayer, mainly differences in cell proliferation, viability, metabolism, morphology, and gene expression, which may affect their function and give different reactions to, e.g., therapeutic agents [24].

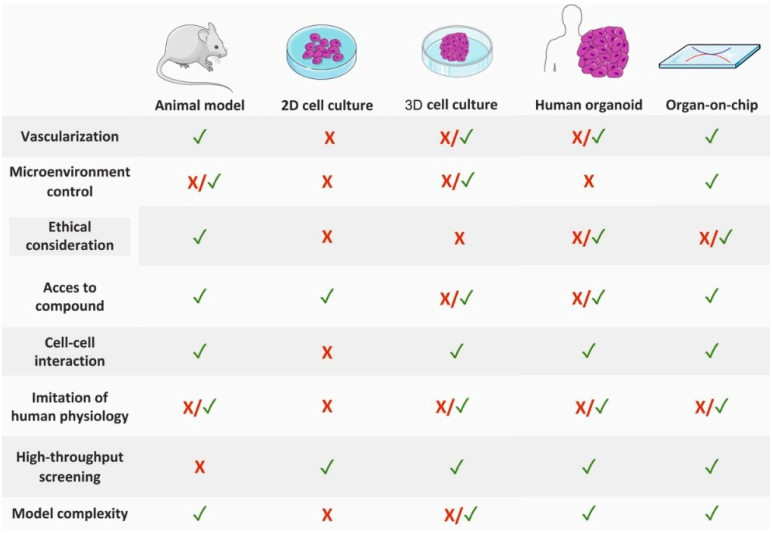
Currently, the research model that is most often used in research due to the possibility of reflecting the conditions prevailing in the body is the animal model. Animal models can be divided into spontaneous (meaning that such animals naturally have human-like diseases) or induced, that is, those in which diseases are artificially induced (e.g., heart disease and cancer) [25–27]. The genomes of humans and animals (e.g., chimpanzees, mice, and pigs) are 85–98% similar [28]. For this reason, animals, and most often mice, are used as a well-established research model in medical experiments to assess the efficacy and safety of therapeutic agents before their introduction to human clinical trials [29]. Unfortunately, even despite this genetic similarity, the animal model responds differently to therapies and may tolerate drugs differently than the patient [30]. For example, in 2014, it was noted that some species of mice are resistant to the Ebola virus while others die. This shows that no single animal model can perfectly reproduce human disease and that differences between strains or species can lead to a misunderstanding of disease development [25].

Moreover, there are many ethical and legal aspects to the use of laboratory animals. According to Humane Society International [31], animals used in research are often subjected to cruel experimental methods that involve inducing wounds, tumours, and inhumane methods of killing. The use of laboratory animals has a great scientific value. For this reason, it is reflected in almost every countries regulations that permit but protect their use [32]. However, wherever possible, laboratory tests on animals are currently pursuing

the introduction of the 3R, which says that animal models could be replaced with other research models such as in vivo conditions.

One of the most popular approaches to replace animal models is three-dimensional (3D) cell culture, which has been shown to reduce the gap between cell cultures and physiological tissues. This is mainly provided through the ability to produce physiologically relevant models by mimicking in vivo microarchitecture, chemical gradients, cell–cell interactions, and cell–environment interactions [33,34]. The most important difference between 2D and 3D culture is the fact that three-dimensional cell structures are cultured in an environment that allows interaction with the external environment in three dimensions (such as in in vivo conditions). Three-dimensional cellular models are obtained by methods that do not require additional scaffolds due to the natural properties of cells, such as the ability to self-aggregate, or by methods based on different types of scaffolds (biological scaffolds, polymeric scaffolds, and encapsulation methods). The most common 3D models that do not require additional scaffolding are spheroids, which are mainly achieved by the limitation of cell adhesion to the culture vessel surface, so cell–cell interactions outweigh cell interactions with the surface [35]. Spheroids are most often used as multicellular tumor spheroids (MCTS) models and can be obtained by several different methods, such as plates with a u-shaped bottom [36,37], rotational culture systems [38], spinner flask bioreactors [39], or a hanging drop [40].

One of the main approaches of tissue engineering is the use of three-dimensional scaffolds. These structures act as a template for cell growth and tissue regeneration and are widely used in transplantology. These artificial extracellular matrices have found wide applications in regenerative medicine due to their numerous advantages, including biodegradability, biocompatibility, and good reproducibility [41]. The use of scaffolds provides appropriate matrices that enable the adhesion and proliferation of cells and the formation of three-dimensional structures. Most often, scaffolds used for the formation of 3D models are divided into two main groups: biocompatible scaffolds and encapsulation methods. Biocompatible scaffolds are three-dimensional, fibrous, porous, or permeable biomaterials that perform two main functions: providing support for cell growth and ensuring the interaction between cells and the proper transport of fluids and gases [42]. Biocompatible scaffolds used for cell research are most often made of polycaprolactone (PCL), poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(lactic acid-co-glycolic acid) (PLGA), polystyrene (PS), Poly(propylene fumarate) (PPF), and poly(dimethyl siloxane) (PDMS) [43,44]. Besides all the advantages of this method, one of the disadvantages is the inhomogeneous distribution of cells [45]. This problem can be partially solved by enclosing the biological material in a hydrogel matrix (encapsulation method), which is a dilute polymer or supramolecular network with a given structure and network properties obtained by intermolecular crosslinks (in the case of a polymer molecular network) or by interfibrillar crosslinks (in the case of supramolecular fibrillar hydrogel networks) [46]. There are many materials such as: alginate, hyaluronic acid, agarose, gelatine, collagen, chitosan, and dextran that can form hydrogels [47,48]. A summary of the existing preclinical models with their advantages and disadvantages is presented on the Figure 1. However, even very complex three-dimensional cell cultures are not able to reproduce the characteristics essential for the functions of organs found in living organisms, e.g., tissue–tissue junctions, nutrient and oxygen gradients, and the mechanical action of the microenvironment [49]. To overcome these limitations, to better mimic the complex microenvironment, imitate the flow, and improve the diffusion of nutrients inside the structures, new solutions based on organoids and Organ-on-Chip approaches are developed.



	Animal model	2D cell culture	3D cell culture	Human organoid	Organ-on-chip
Vascularization	✓	X	X/✓	X/✓	✓
Microenvironment control	X/✓	X	X/✓	X	✓
Ethical consideration	✓	X	X	X/✓	X/✓
Acces to compound	✓	✓	X/✓	X/✓	✓
Cell-cell interaction	✓	X	✓	✓	✓
Imitation of human physiology	X/✓	X	X/✓	X/✓	X/✓
High-throughput screening	X	✓	✓	✓	✓
Model complexity	✓	X	X/✓	✓	✓

Figure 1. The most common cellular models used in biomedical research. The table shows the most important features of the models, their limitations (X) and advantages (✓). Some models partially (X/✓) show certain features. The image contains elements from SMART Servier Medical Art by Servier licensed under a Creative Commons Attribution 3.0 Unported License.

Organoids

Organoids are groups of cells that organize into cell structures such as those found in various organs. The name organoid means "organ-like". In many cases, particular cell structures give organs properties like the organs they are intended to resemble [11]. Organoids are three-dimensional tissue cultures grown from stem cells. For organoids to be able to grow and develop, a specific environment (mainly hydrogels of various kinds [17], Figure 2) is created that allows cells to follow ingrained genetic instructions to organize themselves into a specific structure [12]. Compared to traditional monolayers or spheroids, organoids are more like native organs in terms of gene and protein expression, metabolic function, and tissue architecture.

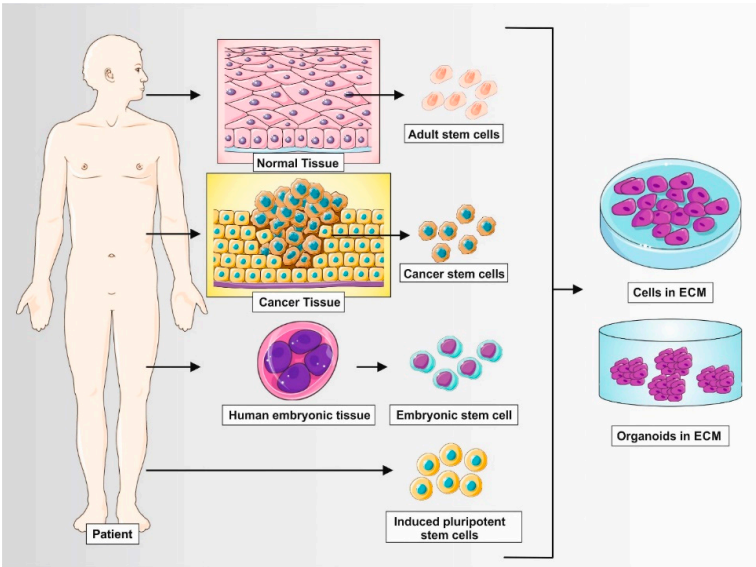


Figure 2. The establishment of human three-dimensional organoids. Organoids can be generated using embryonic stem cells, induced pluripotent stem cells, adult stem cells, or cancer cells in specific 3D ECM-like environment. The image contains elements from SMART Servier Medical Art by Servier licensed under a Creative Commons Attribution 3.0 Unported License.

Stem cells are the main type of cells used for the formation of organoids. Different types of stem cells can be used for organoids formation, depending on what type of organoid is needed. The pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), as well as stem cells that are found in organs, called tissue stem cells or adult somatic stem cells (ASCs), are already used (Figure 2). Types of stem cells differ from each other with growth abilities, limitations, and requirements [12,50]. To form organoids, researchers provide stem cells with conditions that may require specific nutrients, growth factors, signalling molecules, and a physical environment, e.g., protein material on which they can grow. Organoid culture procedures often require the administration of individual ingredients (as mentioned above, specific nutrients, growth factors, and signalling molecules) in a specific sequence and at designated times. The cells culture conditions should promote the multiplication of cells, as well as their differentiation into the types of cells typical for the organ that a given organoid is to represent. Moreover, cells derived from stem cells can self-assemble into cellular structures [19,51]. For example, kidney organoids arise from cells with properties like kidney cells, that organize themselves to form tubules exactly as found in the kidney [15]. The most difficult aspect of forming organoids is finding the proper conditions to promote and stimulate the stem cells. Once the proper conditions are provided for the stem cells, they proliferate, differentiate, form cellular structures, and eventually form the organoids themselves.

Researchers form and use organoids for several reasons. The process of producing organoids that are highly like real organs allows us to understand what external factors cause stem cells to form individual organs at an early stage of human development. In addition, organoids can be used to study diseases or cell signalling. In addition to the external signals that affect stem cells, many internal signals, proteins, and genes that are necessary for cells to produce an entire organ are analysed. These factors are important in understanding how genetic mutations can lead to genetic disease [11,19]. For example, studies using intestinal organoids prepared from materials obtained from six patients with bowel disease led to the discovery of a gene responsible for gut formation [51]. Organoids allow us to study infectious diseases with methods that were not possible before. Brain organoids are used to study the effects of the ZIKA virus on brain development and microcephaly formation [52]. Such research would not be possible using human brain tissue for obvious ethical reasons. In other cases, organoids offer the possibility of testing viral, bacterial, and parasitic infections in ways previously unavailable. An example is the study of the life cycle of the parasite *Cryptosporidium*, which causes a diarrheal disease called cryptosporidiosis [53].

3. Organ-on-Chip

The Organ-on-chip (OoC) technology is based on the use of microfluidic systems made of biocompatible materials to enable the simultaneous cell culture and to reproduce in vivo conditions due to the use of a network of microchambers and microchannels and a laminar flow [22]. In the development of organ-on-chip systems, particular attention should be paid to the appropriate selection of flow conditions (flow rate, frequency of medium change, time of nutrient delivery, and flow direction) to ensure an appropriate level of nutrients and to reproduce blood flow. It is also related to another very important point that is scaling. The size of the organ, flow rates, shear stress values, and the total volume of delivered solutions in the developed device must scale to physiological dimensions [54]. Depending on the type of Organ-on-chip application, it is possible to use various methods and construction materials. Due to numerous advantages, PDMS is the most used elastomer to produce Organ-on-Chip devices. However, it should be remembered that PDMS is not resistant to organic solvents and can absorb small hydrophobics (such as drugs), which may limit its usefulness in pharmaceutical research. Meer et al. in their research showed differences in the adsorption of small drugs molecules between unmodified PDMS, modified PDMS, and a polystyrene tissue culture plate [55]. Therefore, new biocompatible construction

materials are being sought [56]. The materials used to produce Organ-on-Chip systems and the methods of their processing are presented in Table 1.

Table 1. Materials and processing methods used for Organ-on-chip systems.

Materials	Advantages	Disadvantages	Pre-Processing Methods	Ref.
PDMS	• Gas permeable. • Biocompatible. • Optically transparent. • Flexible. • Easily reproduced complex structures. • Hydrophobic (important for 3D culture). • Optimal for real-time imaging.	• Incompatibility with organic solvents. • Strong adsorption of biomolecules.	• Soft lithography. • Micromolding technique. • 3D printing.	[57–59]
Glass	• Transparent. • Optimal for real-time imaging.	• Time-consuming processing methods. • Hydrophobic. • Impermeable to gases. • Complicated structures are difficult to reproduce.	• Photolithography. • Etching. • Laser cutting.	[60–62]
PMMA	• Optically transparent. • More rigid than elastomers. • Low autofluorescence. • Biocompatible.	• Hydrophilic. • Poorly permeable to gases. • Incompatible with most organic solvents.	• Thermal printing technique. • Micromilling. • Laser cutting.	[63,64]
Thermoplastic elastomers (TPE)	• Resistant to pressure and temperature changes. • Chemically stable. • Transparent in the visible range. • Cheap. • Resistant to many organic solvents (chlorinated solvents can dissolve TPE).	• Hydrophilic. • Poor resistance in specific solvents. • Difficult to assemble under room temperature.	• Hot embossing. • Injection casting.	[65–67]
Polystyrene (PS)	• Ease of fabrication/modification (solvent and thermal bonding). • Inexpensive. • Rigid.	• Poor chemical resistance especially to organics. • Susceptible to UV degradation.	• Injection casting. • Laser ablation process (CO_2 laser system). • Micromolding.	[68,69]
Paper	• The cellulose matrix of the paper allows to obtain a porous structure for cell growth in a 3D format. • Cheap. • Biocompatible. • Easily modifiable.	• Poor mechanical properties.	• Inkjet printing. • Solid wax printing.	[70]

The main idea behind the concept of Organ-on-Chip models was to mimic the behaviour of various cells and even entire organs under flow conditions to reproduce physiological or pathological processes [21]. Organ-on-Chip technology has advanced rapidly because its applications in biology and medicine provide tools that are cost-effective, portable, reduce cost and time, and can also conduct in vitro experiments under more controlled conditions [71]. In such models, cultured mammalian cell lines are a simplified model of an organ in the body and are used to assess the cytotoxicity of therapeutic agents and potential pathological agents. The first Organ-on-Chip model that initiated the dynamic development of research in this direction was the Lung-on-Chip [72]. Compared to traditional cultures, the use of flow conditions in Organ-on-Chip systems reproduces a controlled culture microenvironment and the mimicking of tissue functions in terms of physical and chemical signals. Moreover, the possibility of integration of the developed model with analytical methods enables simultaneous cell culture, its real-time monitoring, and the influence of various external factors.

Nevertheless, studies conducted with the use of Organ-on-Chip systems have several limitations due to differences between the native extracellular matrix (ECM) microenvironment and prevailing in the OoC in terms of stiffness, permeability, and biochemical

components. In some cases, due to the specificity of research laboratories and the time-consuming stage of isolating cells from the patient, the differences between Organ-on-chip and in vivo conditions are also related to the use of commercially available cell lines to obtain a three-dimensional model [73]. The organoids themselves also have several limitations, which are mainly due to the random organization of cells, the lack of flow conditions, and the lack of precise microenvironment control. These problems can be solved by a combination of Organ-on-Chip systems with organoids. The combination of flow conditions with the unique biology of the organoids can provide a universal, ideal research tool for screening therapeutic agents and mechanisms underlying organ regeneration. Moreover, the possibility of using stem cells from the patient in the Organoids-on-Chip system is an opportunity to create patient-specific disease models and opens many possibilities for the development of an ideal personalized therapy.

3.1. Tumour

Cancer is still one of the leading causes of human death worldwide [74]. There are many different types of cancer in terms of origin, metabolism, morphology, and drug resistance. Currently, there are many anti-cancer therapies that are bringing better and better results over the years [75]. However, choosing the right drug for a specific type of cancer is often a lottery. In addition to curing cancer, other human organs are also destroyed. Therefore, it is important, especially in the case of cancer diseases, to develop a model that would allow the matching of the appropriate drug outside of the living organism. Cancer research is an area where organoids (tumoroids) can be easily used [76]. Many cancers have cells that behave like stem cells, so researchers can use them to grow tumor organoids (Figure 2). Moreover, the tumoroids can derive from patient tissues [77]. The ability to grow mini tumours that model different tumours allows scientists to analyse tumor development in detail, i.e., understand the genes, proteins, and signalling pathways used by cancer cells, to discover new ways to stop the cancer from growing or metastasizing. Taking a slightly different direction, scientists are also investigating what gene mutations cause tumours to occur in healthy organoids. Because the Organ-on-Chip approach used together with organoids can increase better reproduction of in vivo tumors (flow condition, proper scale of tumour model, and high throughput) and could open a new window to anti-cancer research in personalized medicine, scientists started to produce this type of tool [78]. An example of the use of breast cancer organoids in OoC systems is the work of Shirure et al., who presented a microfluidic platform that mimics the transport of biological mass near the arterial end of the capillary in the tumor microenvironment. The designed platform was used to observe the characteristic features of tumor progression, including cell proliferation, cell migration, angiogenesis, and tumor cell entry. Moreover, the platform brings the opportunity to use it to mimic the physiological delivery of drugs to the tumor through the vascular network to assess the effectiveness of therapy, i.e., in personalized medicine [79]. Rajasekar et al. developed the "IFlowPlate" microfluidic platform capable of simultaneously culturing more than 100 independently vascularized and perfused colon organoids in vitro. The developed platform was used as a model of innate immune colitis where circulating immune cells could be recruited from the vascular network, differentiate into macrophages, and penetrate colon organoids in response to tumor necrosis factor (TNF) stimulation. Due to the ability to vascularize colon cancer organoids under intravascular perfusion conditions, the IFlowPlate platform can provide possibilities for screening new therapeutic agents and modelling disease states [80].

3.2. Lung

Lung diseases are an extremely diverse group in terms of ethology and treatment. They are one of the most common in society, and various factors may be responsible for their formation, including pathogens, genetic, and environmental conditions [81]. For this reason, there is a need to develop diagnostic devices and research models that allow the identification of pathogens and the testing of new therapeutic agents. As previously men-

tioned, Lung-on-Chip was the first microfluidic device to set a new direction in the research of Organ-on-Chip systems. In 2010, authors described a biomimetic microfluidic system made of PDMS with two microchannels separated by a porous PDMS membrane covered with ECM (fibronectin or collagen), in which alveolar epithelial cells and pulmonary microvascular endothelial cells were grown on opposite sides of the membrane [72]. In this way, the functional alveolar–capillary interface of the human lung was successfully recreated. It has been proven that by developing a system that mimics the functions of the epithelial barrier, it is possible to directly quantify the complex biological processes of the pulmonary organ on a single chip, which was not possible with traditional cultures. Since then, research has been conducted on improving the Lung-on-chip device, which, despite its advantages, has not been able to reproduce the physical and biochemical properties of the vesicle basement membrane. In the described Zamprogno et al. [82] work, a stretchable membrane formed by pouring droplets of the CE (collagen–elastin) solution onto a gold mesh was used instead of the PDMS layer. This membrane allowed the reconstruction of the composition and mechanical, biophysical, and transport properties of the lung–alveolar barrier. Nevertheless, there are some disadvantages of Lung-on-Chip systems mainly due to the use of immortalized cell lines instead of the original human cell lines, which is due to the lack of many important structural and immune cells found in the body. To improve the existing models, since 2015, work has also started on the development of a lung organoids derived from differentiated stem cells. Dye et al. [82] developed a method for differentiating embryonic and induced human pluripotent stem cells (hPSCs). The organoids were cultured under laboratory conditions for 100 days and developed into structures that contained many of the cell types found in the lungs. However, it is not clear whether organoids produced by traditional methods (in culture dishes) will be useful for studying the dynamic interactions between cells under physiological conditions (e.g., influenced by blood flow). Moreover, the biggest problem with organoid formation is the lack of control over the organoids' size and cell proportions during self-assembly [83]. The combination of an Organ-on-Chip system with a lung organoid or even obtaining lung organoids in Organ-on-Chip systems could eliminate these limitations, significantly improve research, accelerate the differentiation process, and be a great introduction to personalized medicine.

3.3. Liver

The liver is the largest internal human organ, fulfilling many functions, including the synthesis, metabolism of carbohydrates, proteins, albumin and various hormones, glycogen storage, the detoxification of endogenous and exogenous substances, bile secretion, and the regulation of inflammatory responses [84]. Furthermore, the liver plays a key role in the metabolism and biotransformation of drugs. The liver is formed by parenchymal cells (hepatocytes) and non-parenchymal cells such as endothelial cells, biliary epithelial cells, Kupffer cells, and liver stellate cells (HSCs), which together form a hexagonal unit called a lobule [85]. The current in vitro models are based on the use of mainly one type of cell—hepatocytes—which is a significant simplification of this organ [86]. Therefore, the development of a more advanced liver model is one of the increasingly discussed research problems. A 3D vascularized liver organoid composed of induced liver cells (iHep) and cell-free liver extracellular matrix (LEM) cultured in a microfluidic system that showed improved liver function, metabolic activity, biosynthetic activity, and drug responses was presented by Jin et al. [87]. Wang et al. presented a strategy to produce liver organoids derived from the human-induced pluripotent cells (hiPSC) in perfusion chambers. Liver organoids in perfused cultures showed a better cell viability and a higher expression of endodermal and mature liver genes. In addition, liver organoids showed a marked enhancement of liver-specific functions including albumin and urea production and metabolic capacity, indicating the role of mechanical fluid flow in promoting liver organoid function. Authors also showed a hepatotoxic response after acetaminophen (APAP) exposure in a dose- and time-dependent manner [88].

3.4. Heart

People of all ages struggle with heart disease. Heart diseases in adolescents and children are most often the result of birth defects. However, the causes of heart disease in adults can be excessive stress, poor diet, inactivity, smoking, and age [89]. Various types of heart disease affect the functioning of the body and are very dangerous. Therefore, it is extremely important to study this type of disease in vitro. Skardal et al. created a tool for obtaining a heart model under flow conditions. Heart organoids were formed from induced pluripotent stem cells (iPS) and bioprinted into microreactors using a fibrin/gelatine gel. The produced cardiac organoids showed the expression of cardiac biomarkers and were characterized with a high viability during long-term culture. The heart model was used to study the multiorgan response (Liver–Heart–Lung), which allowed the assessment of more complex integrated reactions where the functionality of one organoid influenced the response of another [90]. However, Yin et al. created a 3D co-culture of liver and heart organoids in compartmentalized flow chambers after forming self-assembled organoids from human-induced pluripotent stem cells (hiPSCs), which they used to evaluate the safety of a cardiac antidepressant after hepatic metabolism [91].

3.5. Brain

Recently, there has been a growing interest in mimicking the structure of such a complex organ as the brain, which is a multiscale system that makes in vitro modelling a particular challenge. Aspects that are a challenge in Brain-on-Chip systems include: mimicking the brain microenvironment, the cellular architecture (the integration of different cell types), the integration of multiple brain regions, and subunits. These problems also result from deficiencies in the brains present state of knowledge. The brain microenvironment, in particular the extracellular matrix (ECM), has not been fully characterized so far. The brain microenvironment includes substances that are not found in most organs and are extremely difficult to imitate in in vivo culture, e.g., glycosaminoglycans (GAGs) such as hyaluronic acid (HA), proteoglycans such as brevikan, neurocan, and phosphakan, growth factors secreted from other cells, and a certain level of fluid (blood and cerebrospinal fluid). Another important aspect is the maintenance of the delicate structure of the brain—most of the currently used culture surfaces are rigid polymers that can affect abnormal migration, proliferation, and the phenotypes of cells in vitro. Currently, a complete Brain-on-Chip platform has not yet been developed, but there are many microsystems modelling individual processes taking place in the brain that can bring us to the closer development of a complete system. The developed platforms most often concern neurons and the process of myelination. Kerman et al. [92] developed a microfluidic system to study the myelination process (oligodendrocyte formation of the myelin sheath around axons) in the central nervous system, which enables a faster and more efficient transmission of information in the brain. In this work, an Organ-on-Chip system was developed for the long-term observation of the myelination process in mouse cells. Agrawal et al. [93] designed a microfluidic system based on platinum electrodes to stimulate rodent neurons with an electric field. Electrical stimulation has been shown to facilitate the maturation of oligodendrocytes and the formation of myelin. As mentioned before, the brain is a very complex structure with not fully understood functions, which makes it difficult to study in model organisms. In addition to developing the Brain-on-Chip model, research is also carried out on the production of organoids derived from human stem cells. Lancaster et al. developed three-dimensional organoids derived from hPSC that corresponded to the functions of various regions of the brain [13]. The example of combination brain organoids and an OoC approach is the work of Wang et al. [94]. They created a brain system of organoids on a chip derived from human-induced pluripotent stem cells (hiPSCs). The produced brain organoids showed well-defined neural differentiation, regionalization, and cortical organization, demonstrating the key features of the early stages of human brain development. Brain organoids exposed to nicotine showed premature neuronal differentiation with increased expression of a neural marker. In addition, brain regionalization and cortical

disruption of nicotine-treated organoids identified by the expression of forebrain, hindbrain, and cortical neural layer markers have been noted. Cho et al. demonstrated combining brain organoids derived from human PSC by reconstructing the 3D brain mimetic microenvironment with the cell-free brain extracellular matrix derived from human brain tissue and dynamic microfluidic microsystems [95].

3.6. Pancreatic

The main direction of research on the pancreas is the study of the endocrine part of the pancreas, i.e., the part responsible for the secretion of hormones and maintaining proper glucose homeostasis in the body. In this part of the pancreas, there are pancreatic islets responsible for maintaining the proper level of glucose in the blood—mainly through the secretion of an appropriate amount of insulin [96]. Therefore, it is extremely important to reproduce the structure of the pancreatic islet, which is a microscopic spherical cluster of five types of cells that synthesize and release hormones. Such systems are based mainly on polymer microsystems with a geometry conducive to the formation of three-dimensional structures and developed for the study of insulin, glucagon, and somatostatin secretion from pancreatic islet cells, and the measurement of mitochondrial potential to assess islet functionality [97]. The main goal of the Islet-on-Chip platforms is to understand the pathology of diabetic disease and discover antidiabetic therapies. The biggest problem in develop Islet-on-Chip model is the imitation of the complicated structure of the pancreatic islet, which, depending on the species, differs in the composition and location of cells [98]. Due to the lack of isolated human pancreatic cells, studies are most often carried out in mouse or rat isolated islet [99,100], ß-cells spheroids [101], or laboratory animals [102].

In the literature, an Islet-on-Chip model consisting of two commercially available rodent pancreatic islet cell lines (α and ß cells), which reproduced the morphology of the rodent pancreatic islet, was presented [103]. The developed model was fully functional in terms of viability, proliferation, secretion of pancreatic hormones (insulin and glucagon), and allowed the continuous monitoring of the condition of the culture and the impact of potential pathogenic and therapeutic agents. In recent years, there have been several studies using stem cells to create organoids of pancreatic islets. This approach is extremely valuable in regenerative medicine and transplantology due to the shortage of donors and problems with transplant rejection. Hirano et al. [104] developed a closed-channel microfluidic platform for the creation and culture of organoids composed of hPSC. In this study the achievement of functional aggregates of endocrine cells that showed the functions of pancreatic islets was confirmed. The culture of organoids in Organ-on-Chip systems offers extraordinary possibilities, mainly due to the possibility of producing multiple islets from patients stem cells and minimizing the risk of transplant rejection. Moreover, this approach can also be extremely valuable in the personalized medicine of pancreatic cancers, which are one of the deadliest cancers worldwide due to their low detectability.

3.7. Others

The above-mentioned examples of the use of organoids in OoC systems are only an illustration of their diversity. Only examples of the most important in vitro organs models produced with the use of these two extremely important biological and engineering tools have been selected and described. However, in the literature there are many other examples of organoids, e.g., kidney [105], retinal [106], or intestine [107] cultured in Organ-on-Chip systems.

4. Conclusions

Organoids are likely to become an indispensable tool for biological research and drug discovery within a few years. Although the production of organoids is a method that allows us to obtain highly advanced in vitro models, it should be remembered that this method is still relatively young. Researchers continue to develop new ways to create organoids that resemble many tissues and organs. The first point that needs improvement

is certainly the increased control over the organoid growth environment. In most cases, the creation of an organoid culture is based on the self-organization of cells. The self-organization process does not allow for the strict control of culture, which leads to a high phenotypic variability of the obtained organoids. The precise characterization of the obtained structure is necessary to introduce these structures to clinical applications. In addition, the reproducibility of the model should be improved, and the culture protocols should be strictly defined to achieve reproducibility. Another aspect is the complexity of such models. It should be considered whether the current methods of creating organoids and the types of cells they consist of ensure an appropriate level of complexity of the developed model. In addition, a very important aspect is to ensure appropriate flow conditions. Static cultures of organoids do not reflect fluid flow, shear stress, and the interaction between tissues and organs. In addition to increasing the variety of organoids, scientists are constantly striving to produce organoids that best represent real organs. The more the organoids resemble the real tissues of individual organs, the more precise the data obtained by scientists becomes. On the other hand, the field of Organ-on-Chip brings new hopes to produce tools allowing for the generation of in vitro models reproducing important in vivo features such as scaling, flow, and multi-cell/multi-organ communication. Therefore, the combination of organoids and Organ-on-Chip systems is an attempt to generally accelerate research works (Figure 3). Most organoids are small, which allows researchers to easily grow multiple organoids at once. The combination of this advantage with modern, high-performance flow technologies allows researchers to test and compare hundreds of materials simultaneously. Since organoids can represent healthy and diseased organs as well as cancer, these high-throughput studies can become highly effective tools for researchers to rapidly test new drugs, medical therapies, and more. The development of organoid cultures and microfluidic OoC systems opens the way to preclinical and personalized research. The use of patient organoids and flow-through tools that mimic the natural conditions of the tissue development environment make them a valuable tool with a high potential to reduce or completely abolish animal testing, which, as mentioned above, can provide erroneous predictions. There are probably many more ways organoids can affect science and medicine, but many of them have yet to be discovered.

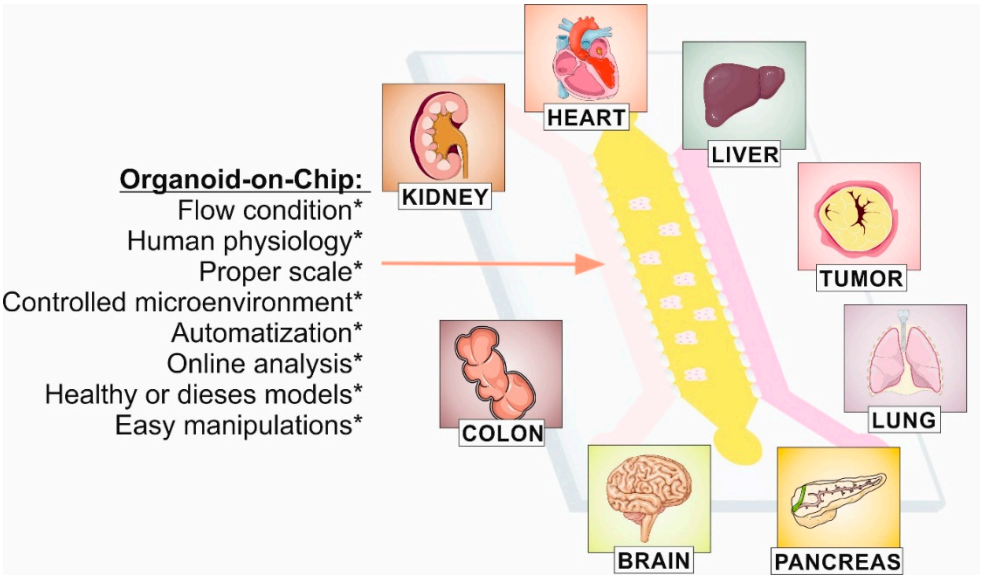


Figure 3. Overview image of different types of Organ-on-Chip devices in which organoids are or can be used. * Possible elements and characteristic of Organoid-on-Chip approach. The image contains elements from SMART Servier Medical Art by Servier licensed under a Creative Commons Attribution 3.0 Unported License.

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