Chapter 1

Organ on a Chip

Burcin Gungor[1]

Abstract

Traditional drug development methods have relied on 2D cell cultures and animal models, which have not been able to adequately represent human biology. Due to genetic and physiological variances in human biology, animal models cannot yield accurate results, whereas 2D cell cultures are insufficient in simulating tissue architecture and cell-to-cell interactions. These limitations have led to the development of organoid technology, which is made from stem cells and imitates the three-dimensional structure of organs. Organoids offer a strong platform for drug testing, disease mechanism modeling, and the creation of individualized treatment plans, but they have drawbacks, including long-term stability and microenvironment control. By incorporating organoids onto microchips, organ-on-a-chip technology has been created, improving biological realism and allowing us to evaluate pharmacological effects more precisely. Organ-on-chip technology replicates the microphysiological characteristics of organs on a chip by using bioengineering methods and microfluidic devices. The study of cancer is among the most notable applications of this technology. By accurately simulating the tumor microenvironment and the interactions between cancer cells and their surroundings, cancer-on-a-chip models help us comprehend the intricate nature of cancer biology. They thus offer a potent platform for the creation of fresh approaches to treatment as well as the assessment of the efficacy of current ones. These models can be used to precisely and thoroughly examine the efficacy of treatment regimens and drug combinations, especially for aggressive disease types like glioblastoma and breast cancer.

Organ-on-chip technologies have revolutionary promise for advancing tailored treatment plans, understanding the mechanisms underlying complicated diseases like cancer, and speeding up medication development. These creative methods are ground-breaking instruments that will influence contemporary medicine and improve human health in the future.

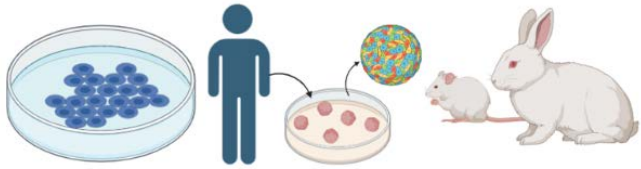
1 Asst. Prof., Department of Biochemistry, Faculty of Pharmacy, Yeditepe University, Istanbul, Türkiye, burcin.gungor@yeditepe.edu.tr, ORCID ID:0000-0001-7725-5835

https://doi.org/10.58830/ozgur.pub1094.c4352

1. Organoids

Organoids are three-dimensional (3D) biological entities that are produced in vitro from stem cells. These small, organ-like structures may replicate organogenesis processes, exhibit tissue-specific cell variety, and replicate organ functions-all of which have the potential to mimic human biology (Lehmann et al., 2019). Because they lack cellular connections and natural tissue structure, traditional animal models and two-dimensional (2D) cell cultures have hindered study in this area. However, organoids have given science a new lease on life. These organoids have great promise for a variety of applications, including drug screening, tailored therapy, and the modeling of genetic disorders (Clevers, 2016).

2D cell cultures and conventional animal models have been for years crucial for understanding cellular processes and drug development research; however, it is well known that these systems fall short of accurately representing human biology. The transferability of data collected in animal models to therapeutic applications is limited by interspecies genetic, physiological, and metabolic variations resulting from human biology. Results from animal models do not always translate to human outcomes, particularly in drug development processes. The associated 3D structure of tissues and the intricacy of cellular interactions cannot be adequately reflected by 2D cell cultures, despite the fact that they offer an appropriate platform for studying cellular processes in a more controlled environment. The microenvironment created when cells grow on a flat surface lacks cell-matrix interaction, giving rise to a structure that is very different from the physiology of natural tissues.



	2D Cell Cultures	3D Organoid Cultures	Animal Models
Vascularization	Limited	Limited	Feasible
Biobanking	Feasible	Feasible	Feasible
High-througput screening	Applicable	Applicable	Not Applicable
Modeling organogenesis	Not Applicable	Suitable	Not Suitable
Modeling patient-derived organoids	Not Applicable	Feasible	Poorly Feasibel
Manipulation	Feasible	Feasible	Limited
Modeling for human physiology	Limited	Feasible	Feasible
Reproducibility	High	Low	Low
Heterogeneity	Low	High	High
Modelling cellular communications	Feasible	Feasible	Limited

Figure 1. Advantages and limitations of 2D cell cultures, animal models, and 3D organoid cultures. Organoids offer significant advantages over traditional 2D cultures and animal models. This makes them an ideal platform for performing a variety of experiments, modeling human diseases, and performing high-throughput drug screens.

Three-dimensional (3D) organoid models developed to overcome these limitations stand out as innovative systems that complement the shortcomings of traditional modeling approaches (Heydari et al., 2021), (Kim et al., 2020) (Figure 1). These innovative models are becoming increasingly important in fields such as organogenesis, disease modeling and medicine. development that offers a higher level of physiological realism at both the cellular and tissue level.

1.1. Historical development of organoids

Innovations in technology and biological research have affected the evolution of organoids over time. Henry Van Peters Wilson showed the cells' ability to self-organize in 1907 when he found that isolated sponge cells could self-organize and regenerate a whole organism. Malcolm Steinberg proposed the "differential adhesion hypothesis" in 1964, contending that various surface adhesions may account for cell organization.

The isolation of pluripotent stem cells (PSCs) from mouse embryos in the 1980s and the discovery of human embryonic stem cells in 1998 gave impetus to organoid research. The development of iPSC technology in 2006 created a major revolution in mimicking embryonic development processes. During the same period, three-dimensional culture media such as ECM and Matrigel enabled cells to organize similarly to their natural environment.

In 2009, the foundations of modern organoid studies were laid when Hans Clevers and his team derived organoids from mouse intestinal stem cells. This work created a growth media that allowed stem cells to self-organize, and other organoid types were subsequently able to use this technique. A new age in biomedical research has been made possible by organoid models made from human stem cells, such as the brain, liver, and pancreas (Zhu et al., 2024), (Corrò et al., 2020), (Han et al., 2022) (Figure 2).

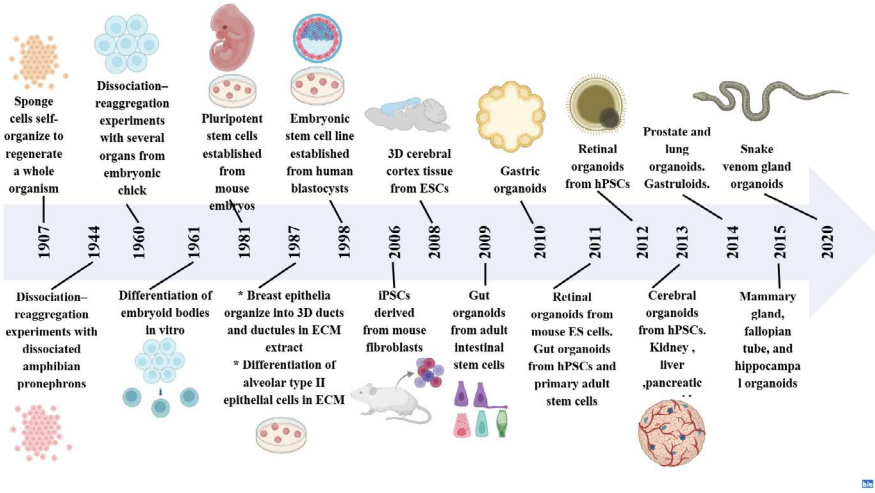


Figure 2. Timeline of organoid technology development. It provides a summary of important breakthroughs and fundamental studies in this field.

1.2. Derivatization of organoids

The mechanism by which stem cells self-organize and create 3D structures under specific circumstances is the foundation for the derivatization of organoids. The characteristics of the organoid are determined by the cellular resources, the signals given, and the culture techniques employed in this procedure. Because organoids mimic how tissues form in nature, they are a valuable tool for understanding disease causes, medication testing, and tissue engineering.

Different culture methods are used in organoid derivation. 3D matrix culture allows organoids to grow in a supportive environment. Natural extracellular matrix (ECM) proteins or synthetic matrices can be used for this purpose. Intestinal and gastric organoids, in particular, are often derived successfully in this method. Suspension cultures, on the other hand, allow cells to grow in free suspension without or with ECM proteins. Optic cup and cerebellar organoids can be derived by this method. Additionally, structures such as kidney organoids can be obtained from cell pellets using the air-liquid interface culture method (Rossi et al., 2018). The development of organoids depends critically on the balance of endogenous and external signals. While some organoids need exogenous cues to self-organize, others can do it solely through endogenous signals. For instance, human stomach organoids first differentiate by foreign cues and then self-organize by endogenous mechanisms, but mouse optic cup organoids only organize by endogenous signals. Kidney organoids and other structures need continuous external stimulation. These signals enable cells to construct the ultimate organoid structure by transitioning from a homogeneous population to an asymmetric organization (Rossi et al., 2018).

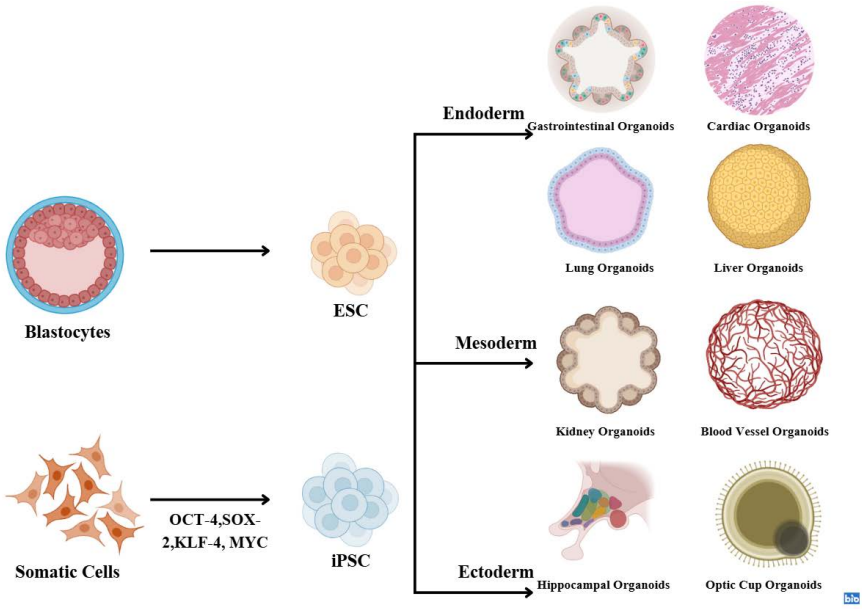


Figure 3. Organoids derived from pluripotent stem cells (PSCs) are created using embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Somatic cells can be reprogrammed into iPSCs with the help of transcription factors. Blastocysts differentiate into ESCs and form three germ layers (endoderm, mesoderm, ectoderm).

The starting cell type is an important factor in organoid derivation. The process, which begins with a single cell, involves cells organizing over time from a homogeneous population into complex structures (Rossi et al., 2018). For example, intestinal organoids are derived this way, starting from adult stem cells. Alternatively, the method of starting from a homogeneous aggregate of cells employs self-patterning mechanisms, as in optic cup organoids. Culturing different cell types together allows complex structures such as liver organoids.

There are several different sources of stem cell-derived organoids, including adult stem cells (AdSCs), induced pluripotent stem cells (iPSCs), and embryonic stem cells (ESCs). The benefits and drawbacks of each kind of stem cell in organoid formation vary.

The blastocyst contains embryonic stem cells (ESCs), which can develop into the endoderm, mesoderm, and ectoderm germ layers (Thomson et al., 1998). The development of diverse organoids, including the lung, liver, kidney, blood arteries, etc., is the result of these differentiation processes. Early organogenesis processes are particularly studied using ESC-derived

organoids, which are crucial to comprehending human developmental biology. The process of genetically reprogramming cells to become pluripotent again is necessary to produce iPSCs from somatic cells. Usually, reprogramming factors like Oct4, Sox2, Klf4, and c-Myc are used to carry out this process (Teshigawara et al., 2015), (Tang et al., 2022), (Balistreri et al., 2020). These factors cause the cells to revert to their embryonic stem cell state and lose their differentiated identity. By cultivating reprogrammed cells under the right circumstances, they can develop into distinct germ layers and, consequently, different organoids (Figure 3).

Because they can be produced using quick and easy procedures, organoids grown from adult stem cells (AdSCs) provide a useful substitute. These organoids have characteristics that are similar to those of adult tissues and are useful in research on viral infections, tissue regeneration, and repair. They are useful for both fundamental research and therapeutic applications due to their long-term genetic stability. By adding certain growth factors, AdSC-derived organoids may be grown in vitro for extended periods of time (Kim et al., 2020), (Tang et al., 2022).

However, AdSCs' restricted ability to differentiate results in these organoids often concentrating on a single cell type (Figure 4). Although this could lessen the variety of organoids, these structures' resemblance to adult tissues offers a significant benefit, particularly when simulating adult tissue regeneration and illness (Loya, 2014), (Hammond-Browning, 2012). AdSCs derived organoids are a valuable tool for therapeutic applications and medical research because of these features.

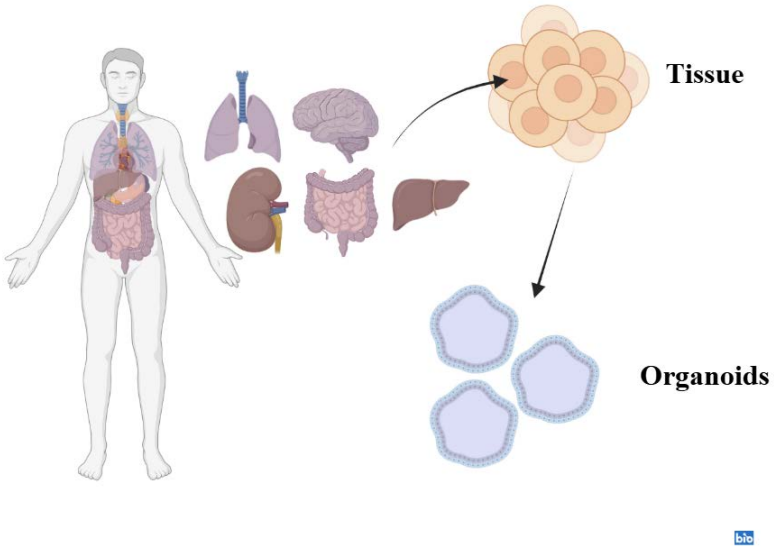


Figure 4. The process of deriving organoids from adult stem cells (AdSCs) begins with undifferentiated cells obtained from specific adult organs. AdSCs found in organs such as lung, brain, liver, intestine and kidney can form organoids by showing self-organization ability in vitro when appropriate culture conditions are provided.

1.3. Establishment of Organoid Cultures

By forming miniature models of biological systems, organoid cultures are an important instrument for researching disease mechanisms and comprehending human biology. Various cell sources are significant in this process. Organoids can be tailored to represent unique biodiversity using tissue-derived cells obtained from human or animal tissue and organ biopsies. Furthermore, organoids can differentiate into any desired cell type thanks to induced pluripotent stem cells, which are cultivated in a lab setting and have an infinite capacity for proliferation (Clevers, 2016). Particularly, cancer stem cells are favored for use in cancer research and for modeling tumor biology.

Successful development of organoids requires the use of soluble factors that promote the growth and differentiation of cells. These factors consist of growth factors and small molecules that direct the transformation of cells into a particular phenotype. While signaling molecules such as Wnt, EGF, HGF, BMP and TGF are important for adult stem cells, factors such as Activin-A, BMP4 and VEGF are used in pluripotent stem cells. These

biochemical signals provide the microenvironment required for cells to form complex organoid structures (Yi et al., 2021) , (Zhao et al., 2022).

The matrix is another key component utilized in organoid cultivation. These matrices are typically composed of natural or synthetic materials and offer a supportive physical environment for cells to build three-dimensional structures. Extracellular matrix components like collagen and Matrigel are examples of natural materials; yet, because of their adaptable topologies, synthetic hydrogels are frequently utilized in organoid creation (Aisenbrey & Murphy, 2020). With characteristics like rigidity and biochemical content, the matrix can control cell behavior in addition to ensuring cell adherence and organization.

Physical clues are also of critical importance for organoids to achieve a functional structure. Extracellular matrix support provides cells with both a mechanical structure and biochemical signals. At the same time, the physical structure of the matrix allows nutrients and wastes to move freely between cells, which supports the healthy development of organoids. Creating a dynamically tunable microenvironment enables organoids to mimic and thrive in their natural environments.

Using integrative cues to improve the biological functionality and structural integrity of organoids is the last stage of organoid engineering. By arranging organoids in a particular order, bioprinting technologies make it possible to create functioning tissues (Murphy & Atala, 2014), (Brassard et al., 2021). Additionally, by putting organoids on a microchip, organ-on-a-chip devices can simulate particular organ systems. By merging several organoids, these systems provide ground-breaking potential in fields like drug development and disease modeling and allow the study of intricate biological processes.

The combination of all these processes requires a holistic approach to establishing organoid-based cultures. Cell sources, biochemical and physical factors, supporting matrix and advanced engineering techniques enable the creation of small yet powerful models to understand and simulate human biology (Figure 5). These technologies are becoming increasingly important in modern biomedical research and contribute to the development of new solutions for human health.

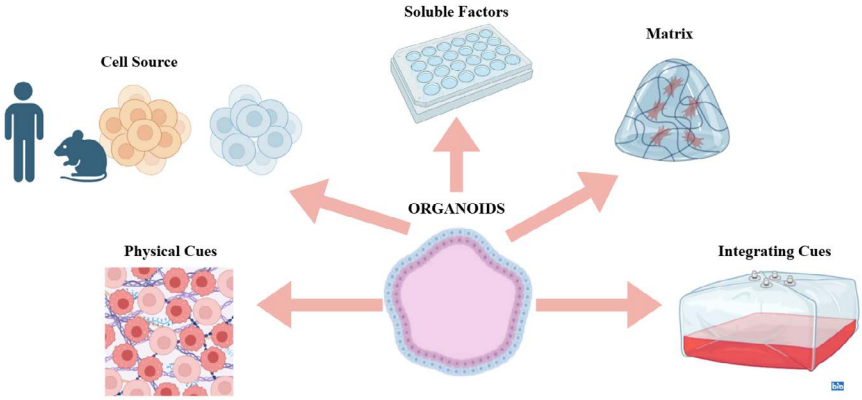


Figure 5. Organoid engineering components. Establishing organoid-based cultures relies on the successful integration of cell sources, soluble factors, matrix, and physical cues.

1.4. Applications of Organoid Technology

3D cell cultures known as organoids that replicate the composition and capabilities of human organs may now be produced in the lab thanks to developments in stem cell culture. Numerous biomedical research fields can benefit from this novel technique, including disease modeling and mechanism study, drug development and toxicity testing, genetic illnesses and personalized medicine, infectious diseases and microbiological research, cancer research, and gene repair (Figure 6).

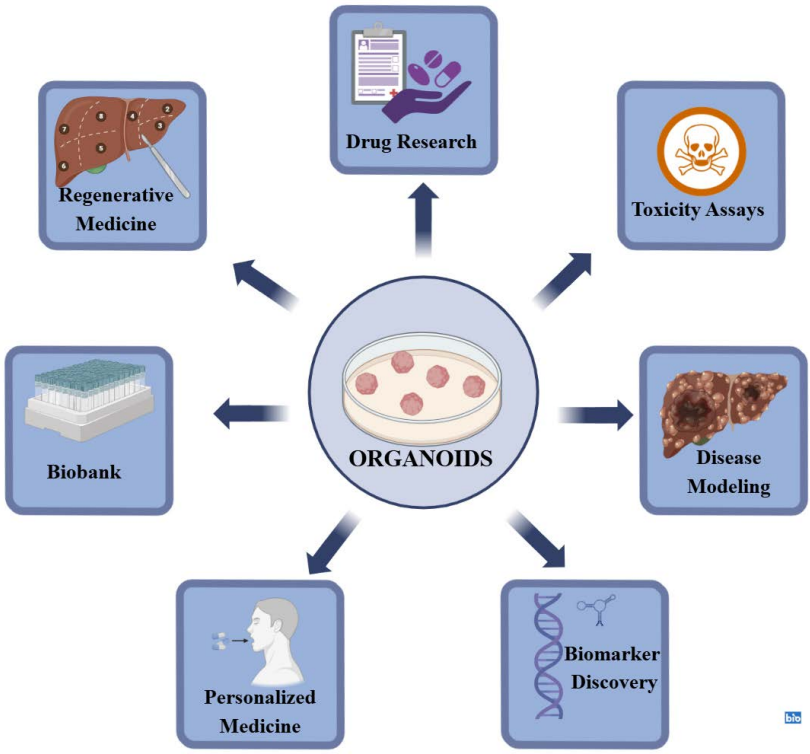


Figure 6. Various applications of Organoid technology

1.4.1. Drug discovery

The drug discovery process suffers from by limitations like patient diversity, uncertain results, and time-consuming drug testing. Organoids now more accurately depict human tissues and physiology, providing a significant substitute in the processes of drug development and toxicity evaluation. The benefits of this technology allow for more accurate testing of new medications' efficacy and safety.

Organoids, which mimic the genetic features of tumors, are used to more precisely assess the effects of certain medications, especially in cancer research. Organoids made from the cells of patients with genetic abnormalities, such colon cancer, for instance, provide effective resources for finding novel medications to treat cancer (van de Wetering et al., 2015), (Lancaster & Huch, 2019). Similarly, antiviral medications for the treatment of such illnesses can be quickly investigated by modeling infections like the Zika virus using brain organoids (Lancaster & Huch, 2019).

Organoids also make it easier to develop personalized medications. Individualized therapy options can be rapidly assessed by modeling diseases based on a person's genetic makeup. Organoids of genetically distinct people can be used to create more specialized and efficient treatment plans. This has a lot of promise, particularly for people with genetic abnormalities and unusual disorders. Organoids therefore provide significant promise for improving drug discovery speed, accuracy, and efficiency.

1.4.2. Toxicity assessment

Organoids have improved drug safety evaluations in toxicology research by offering microenvironments that are more similar to human physiology. Due to their crucial roles in drug metabolism and excretion, the liver and kidney are susceptible to damage from drugs. Due to limited cellular interaction or biological differences between species, traditional models, such as 2D cell lines and animal models, may not accurately represent human physiology (Tang et al., 2022), (S. Yang et al., 2023). This reduces the preclinical test results' confidence.

Organoids address these shortcomings and provide useful substitutes in toxicological research. For instance, high levels of CYP enzyme expression in liver organoids improve hepatotoxicity tests. An essential technique for evaluating hepatotoxicity is liver organoids. Key aspects of liver metabolism, including the production of CYP enzymes, are represented by intrahepatic cholangiocyte organoids (ICOs) generated from human AdSCs that were produced under circumstances of differentiation into the hepatic lineage. Shi et al. showed that ICOs are a practical ex vivo model for evaluating bile cytotoxicity. In this study, the relationships between necroptosis and biliary tract disease were defined with the help of ICOs (Shi et al., 2022). In nephrotoxicity evaluations, kidney organoids are perfect for tracking changes in cell viability and gene expression. Gu et al. tested the possible nephrotoxic effects of Esculentoside A using kidney organoids generated from iPSCs. This study showed that exposure to Esculentoside A results in morphological abnormalities, altered gene expression patterns, and decreased cell viability. These findings support the notion that kidney organoids are a viable method for assessing a compound's harmful effects (Gu et al., 2023).

In addition, 3D skins models provide an innovative approach to toxicity testing in the pharmaceutical and cosmetics sectors. Reconstructed epidermis and full-thickness skin are examples of artificial models that accurately represent both healthy and pathological skin situations. These models are widely used and are continuously being refined, adding new layers like

immune cells, despite the limitations on animal testing (Caipa Garcia et al., 2022).

1.4.3. Cancer studies

Organoid technology offers a significant innovation in understanding cancer biology and developing treatments. Organoids derived from patients overcome the limitations of traditional models by accurately mimicking the genetic characteristics and microenvironment of tumors. Animal cancer models, human cancer cell lines, or primary patient-derived tumor xenografts (PDXs) are tools used in cancer research. However, these methods were insufficient to accurately represent the biology and pathophysiology of the host tumor (H. Xu et al., 2018). Cancer cell lines include primary (originating from patients) and immortalized tumor cells. While primary cancer cells retain the characteristics of the original tumor to a limited extent, their short lifespan and slow growth limit research capacity. Immortalized cell lines, despite the advantage of unlimited proliferation, may fail in phenotypic representation by losing genetic diversity in long-term cultures. 2D culture systems are also inadequate to mimic in vivo conditions and cannot accurately reflect tumor heterogeneity. PDX models are created by transferring patient-derived tumors to animals, but have limited use due to interspecies biological differences and high cost (S. Yang et al., 2023), (Fan et al., 2019).

Organoid technology provides a potent substitute to get beyond such barriers. Cells from surgical samples or biopsy material are cultivated in a three-dimensional matrix to create a model that replicates the natural tumor microenvironment. By reflecting the tumor's genetic makeup and drug response in a patient-specific way, this technique makes individualized treatment methods possible. Especially in examining metastasis, the mechanisms of spread of tumor cells to other tissues can be analyzed through organoids (Lo et al., 2020). Ultimately, organoid technology makes it possible to better understand tumor biology in cancer research and develop more effective, personalized treatment strategies.

2. Development of Organ on a Chip

The drug development process is considered one of the most complex and challenging areas of biomedical research as it takes a long time and requires high costs. The failure rate in the procedure is more than 80%, even though only a small percentage of medication candidates investigated in preclinical stages pass clinical trials. The failure of medication candidates owing to toxicity (30%) and ineffectiveness (60%) are the primary causes of

this state of affairs (X. Xu et al., 2024). Conventional modeling techniques are insufficient to address these issues. 2D cell cultures are inadequate for simulating the natural microenvironment and tissue architecture of cells, yet animal models cannot accurately represent human biology because of biological variations between species. Organ on a chip (OoC) technology, developed as a solution to these problems, has the potential to model human biology more accurately.

OoC technology are microengineering platforms that can mimic the functional and structural features of human organs in a laboratory environment. Combining fields like microfluidic systems, bioengineering, stem cell technologies, and bioprinting produced this ground-breaking breakthrough. OoCs are small-scale biomimetic devices that incorporate human cells and are usually made of biocompatible and flexible materials (such as poly(dimethylsiloxane)). These systems offer a unique platform that mimics the physiological circumstances of actual organs by simulating the microenvironment and functioning of one or more organs in vitro. Microfluidic channels that replicate the natural milieu of cells and tissue organizations are employed in the creation of OoC. These channels are made to resemble biological functions like blood flow and the movement of nutrients and oxygen. Oxygen and nutrients are continuously supplied by the fluid flow, which also eliminates waste products and metabolites from the cells. Physical stimuli like fluid shear force also encourage the histochemical differentiation of several cell types, including endothelial and epithelial cells (Whitesides, 2006), (Driver & Mishra, 2023). The main biological elements utilized in OoCs are immortalized cell lines and human-derived stem cells (Park et al., 2019). More accurate and trustworthy outcomes in drug development, toxicity testing, disease modeling, and precision medicine have been made possible by OoC models (H. Wang et al., 2024). This technology has become a ground-breaking tool in biomedical research because it more accurately replicates human biology.

2.1. Application areas of Organ-on-a-Chip

One particularly noteworthy instrument in the drug research and discovery process is OoC technology. Accurate modeling of drug absorption, distribution, metabolism, and excretion (pharmacokinetics) and their effects on target organs (pharmacodynamics) is made possible by these systems, which replicate the biological, chemical, and mechanical processes of human organs at the microscale. In addition to being a potent platform for personalized medicine, genetic disease research, and cancer studies, OoC offers quick and accurate results in crucial procedures including

toxicity assessment and efficacy analysis (Figure 7). This method saves time and money in preclinical testing, which improves the efficiency of drug development procedures (Joseph et al., 2022).

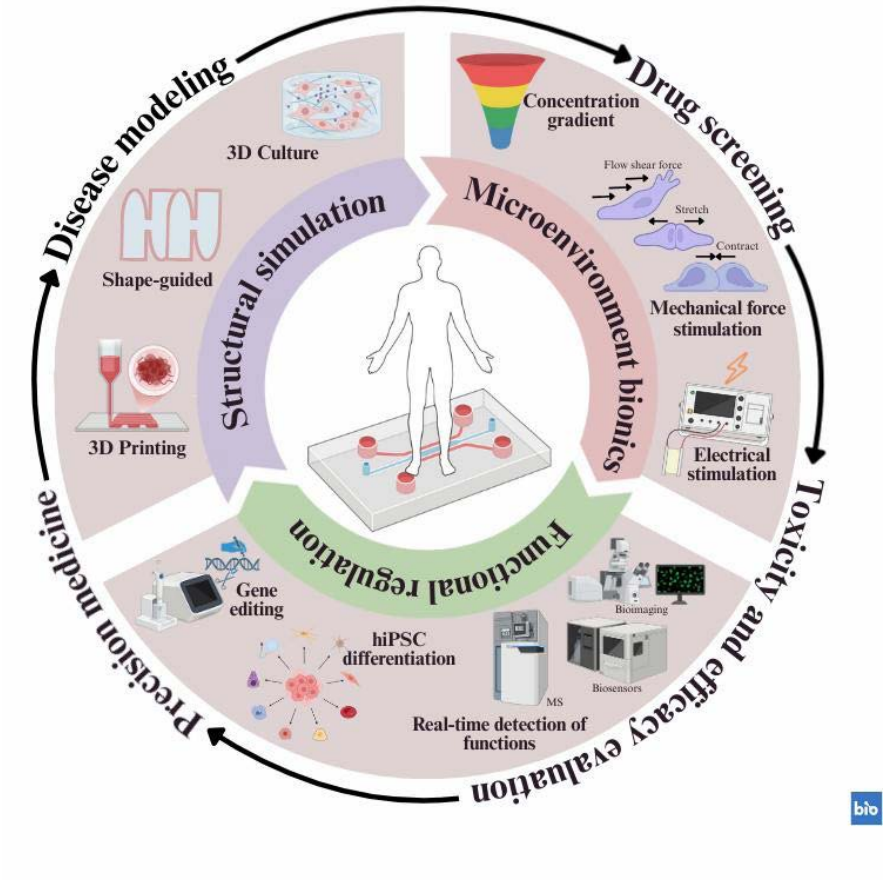


Figure 7. Scheme of application areas of Organ-on-Chip technology

2.1.1. Drug discovery

From the discovery stage to the drug's release onto the market, the development of new medications necessitates a very high time and resource commitment. About 90% of medication candidates that make it to clinical trials fail, which costs the industry a lot of money and delays patients' access to new therapies (Zhai et al., 2019). Among the most important processes in the drug development process is the selection of the best compounds based on factors including toxicity, efficacy, and safety. Prior to human

clinical trials, one of the most important strategies to expedite the process and cut expenses is to have a thorough and accurate screening procedure (Y. Wang et al., 2023).

Traditional drug screening methods generally rely on animal-based in vivo models and cell-based in vitro systems. However, these methods offer limited predictive ability because they cannot adequately mimic human biology. Recent advances in microfabrication and tissue engineering have enabled the development of OoC technology as an innovative solution to overcome these limitations. OoC mimic the in vivo structure and function of human organs, eliminating interspecies differences and providing higher accuracy in preclinical assessments such as drug metabolism, toxicity, and efficacy (Liu et al., 2019). For example, chips that mimic specific organs, such as the liver or kidney, can be used to study the effects of drugs at the organ level, while by integrating multiple organ chips, the systemic effects and interorgan interactions of drugs can be comprehensively analyzed.

2.1.2. Toxicity assessment

Accurately predicting drug-induced toxicity is one of the most difficult tasks in the drug development process. Interspecies differences limit the ability of traditional animal-based models to forecast toxicity, which sparks ethical debates. By utilizing human cells and avoiding interspecies variations, OoC systems improve the ability to anticipate toxicity. Particularly useful for analyzing the harmful effects of medication metabolic byproducts are liver and kidney chips. For example, in 2009, Toh and his team developed a microfluidic liver cell chip to evaluate drug toxicity in a laboratory setting. This device incorporated multiple cell culture chips and a linear concentration gradient generator. Its structure, designed with microcolumns, allowed hepatocytes to settle in a central cell chamber and perform their metabolic activities. The researchers tested five hepatotoxic drugs, including paracetamol, on this chip and successfully obtained in vitro toxicity data consistent with in vivo findings. Such innovative approaches make the drug safety assessment process in preclinical testing more precise while reducing reliance on animal models (Toh et al., 2009), (Z. Li et al., 2022).

2.1.3. Cancer

OoC systems offer a significant benefit for comprehending the intricacy of cancer biology and creating novel therapeutic strategies. An excellent platform for assessing the efficacy and adverse effects of cancer medications is provided by tumor-on-a-chip models, which replicate the cancer microenvironment and the interactions of tumor cells with surrounding tissues. Furthermore,

these models offer a deeper comprehension of intricate biological processes as immunotherapies, tumor microenvironment, and cancer metastasis. This is seen as a major development, particularly for applications in customized treatment (Sontheimer-Phelps et al., 2019).

For instance, Ingber and friends created a chip for lung tumors in order to study cancer. This chip demonstrated that tumor cells were restricted to smaller regions during simulated lung breathing movements, but that the cells spread when the movements were stopped. Furthermore, orthotopic non-small cell lung cancer growth, treatment responses, and tumor dormancy mechanisms were investigated in vitro using the model built on a microfluidic platform. According to research, tumor cells occupy the alveolar space, decreasing the lung's respiratory motions. This creates a positive feedback loop that encourages tumor development and invasion. Additionally, co-culturing tumor cells and alveolar epithelial cells has been shown to enhance cell-cell interactions; however, endothelial cells may counteract this impact. These results clearly demonstrate the promise of OoC systems in cancer research by improving our knowledge of the dynamics of the tumor microenvironment (Hassell et al., 2017) (Liu et al., 2021).

2.1.3.1. Cancer on a Chip

Being one of the diseases with the highest death rates in the world, cancer places a significant strain on healthcare systems. Uncontrolled cell growth, proliferation, and invasion of surrounding tissues as a result of genetic abnormalities and epigenetic modifications are hallmarks of cancer. Through a process known as metastasis, tumors can spread to other parts of the body during this unchecked growth, making therapy considerably more challenging. Even with the recent development of novel techniques including immunotherapies, targeted treatments, and integrated therapy approaches, comprehending and managing the intricate molecular makeup of cancer remains a significant scientific problem. It is known that the complex biochemical and physical environment surrounding tumor cells, called the tumor microenvironment (TME), plays a major role in the development of cancer, as well as genetic mutations. TME is a dynamic structure containing immune cells, fibroblasts, vascular structures and extracellular matrix (ECM) and not only supports tumor growth but also directs the processes of drug resistance and metastasis. However, traditional in vitro and in vivo models have limitations in understanding the molecular mechanisms of cancer and its interactions with the TME. The intricacy and physiological characteristics of the human tissue microenvironment are not reflected in current systems, despite the fact that transgenic mice and immunodeficient animal models

have been utilized to research some forms of cancer. The creation of novel cancer treatments is severely limited by this deficit (Sontheimer-Phelps et al., 2019), (C. Li et al., 2023).

To overcome these limitations, one of the most innovative approaches that has attracted the attention of the scientific community in recent years is "organ-on-a-chip" technology. OoC has the capacity to mimic the biological and mechanical dynamics of complex microenvironments by bringing together different cell types. The biggest advantage of this technology is that it can successfully model biochemical and mechanical microenvironments that traditional cell culture methods cannot reproduce. Thus, it more accurately reflects the complexity of the tumor microenvironment, providing more realistic data about the behavior of cancer cells. Numerous applications for cancer biology and therapeutic research are available using OoC technology. For instance, the intricate relationships between breast cancer cells and the surrounding tissues are being studied using breast tumor models-on-a-chip. These models are an effective way to assess how medications affect the tumor and create more targeted treatment plans. In a similar vein, glioblastoma-on-a-chip models serve as a valuable resource for comprehending the intricate characteristics of this aggressive brain tumor as well as its resistance to treatment. These applications show how flexible OoC platforms are for various cancer kinds and how useful they can be for research procedures. (Sontheimer-Phelps et al., 2019), (C. Li et al., 2023), (Nejati et al., 2025).

Breast tumor on a Chip

One of the most prevalent cancers in women globally, breast cancer accounts for a sizeable portion of cancer-related fatalities. Genetic and environmental variables interact to cause breast cancer, which is a complicated process with a high degree of molecular and morphological variety. There are various subtypes of breast cancer. Hormone receptor positive (HR+), HER2 positive (HER2+), and triple negative breast cancer (TNBC) are the most prevalent subtypes of these (Firatligil-Yildirir et al., 2023). The treatment process might be difficult because each subtype has unique biological characteristics and therapy responses. Comprehending intricate processes such tumor invasion, metastasis, and medication modes of action is necessary to comprehend the course of breast cancer. These processes have traditionally been modeled using animal models and 2D and 3D cell cultures. These methods, however, are unable to forecast the actual effects of medications in humans and cannot accurately capture the biochemical and physical characteristics of the natural TME. As of right now, a solution that can accurately replicate the tumor microenvironment is breast tumor on a

chip (Figure 8). These models offer a perfect platform for researching how cancer cells proliferate, spread, and react to medications. Additionally, these systems provide a distinct advantage over other conventional techniques due to the controllability of fluid flow, shear stress, media and gas supply, and biochemical gradients (Firatligil-Yildirir et al., 2023), (Subia et al., 2021) (Moccia & Haase, 2021).

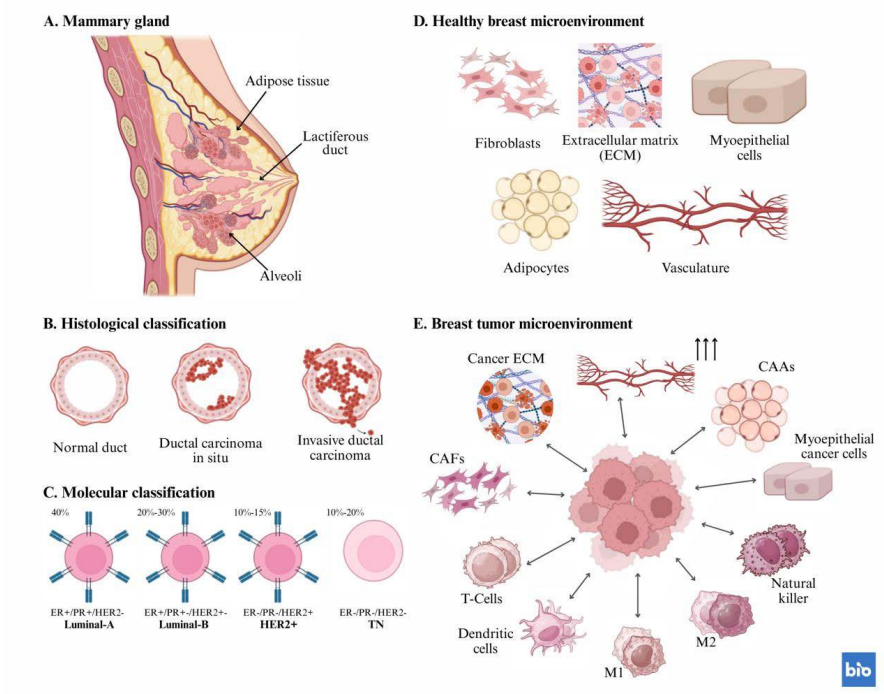


Figure 8. Breast cancer classification and breast-specific tumor microenvironment. (A) Graphical representation of the mammary gland. (B) Histological classification of the breast cancer subtypes. Magenta represents cancerous cells. (C) Molecular classification of the breast cancer subtypes demonstrating their frequency and commonly associated markers. (D) Schematic representation of components of the most abundant healthy mammary gland microenvironment. (E) Components often transformed in the breast tumor microenvironment (modified from Moccia & Haase, 2021).

Glioblastoma on a Chip

One of the most lethal and aggressive brain tumors in the central nervous system is glioblastoma multiforme (GBM). About 17% of all brain tumors are this type of malignant tumor that develops from astrocytes. Due to its extremely invasive nature, GBM spreads quickly to nearby tissues, limiting the effectiveness of conventional therapeutic methods and surgical

procedures. Patients often only live for 16–21 months following diagnosis, and recurrence rates are rather high. Treatment for GBM is made even more challenging by treatment resistance, the intricate complexity of the tumor microenvironment, and the blood-brain barrier's (BBB) ability to block medications from entering the brain (Silvani et al., 2021).

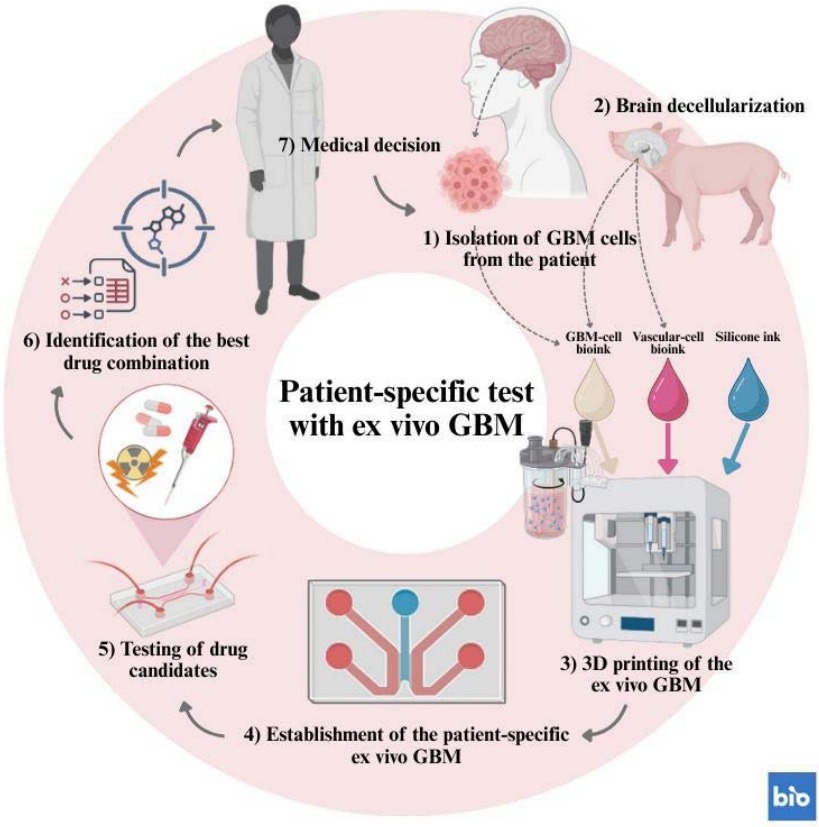


Figure 9. Patient-derived GBM cells are used to produce a GBM-on-a-chip model using pig BdECM bioink. The cells are then cultivated for one to two weeks, and the effectiveness of drug combinations is tested to design a personalized treatment plan (modified from H.-G. Yi et al., 2019).

A glioblastoma-on-a-chip model that can accurately replicate the biochemical and biophysical characteristics of GBM has been created in order to get around these difficulties. First, GBM cells are isolated from the tumor tissue surgically removed from the patient (Figure 9, 1). These cells form the basic building block of the individualized model. The decellularized extracellular matrix (BdECM) bioink from pig brain is then made (Figure 9,

2). Because BdECM replicates the mechanical and metabolic characteristics of the natural tumor microenvironment, it improves the biological fidelity of the model. 3D bioprinters are used to print this bioink once it has been mixed with patient-derived cells. Additional materials like silicone-based inks and bioinks with vascular cells are used in the printing. process to mimic biological processes like oxygen gradients and heterogeneous structure in the tumor microenvironment (Figure 9, 3). The tumor model made on the chip is cultivated for one to two weeks following printing (Figure 9, 4). GBM cells develop in an environment that closely resembles the tumor's clinical characteristics during this phase, and microenvironmental interactions become clearly visible. The cultured model is used to test different combinations of potential drugs (Figure 9, 5). The purpose of these tests is to assess how each treatment option affects the tumor. The most effective combinations are identified as a consequence of the investigation (Figure 9, 6). In particular, the responses of patient-derived tumors to clinical treatments such as temozolomide (TMZ) and concurrent chemoradiation (CCRT) can be modeled on a chip to obtain data compatible with clinical results (H.-G. Yi et al., 2019).

Chip-based glioblastoma models offer a special tool for comprehending tumor biology as well as creating novel medications and therapeutic approaches. Clinical decision-making and the development of individualized treatment plans can both benefit greatly from these models. These modern platforms are regarded as a revolutionary approach in patient-centered treatment procedures and scientific research for cancers that are resistant and lethal, like GBM (H.-G. Yi et al., 2019).

References:

Lehmann, R., Lee, C. M., Shugart, E. C., Benedetti, M., Charo, R. A., Gartner, Z., Hogan, B., Knoblich, J., Nelson, C. M., & Wilson, K. M. (2019). Human organoids: a new dimension in cell biology. Molecular Biology of the Cell, 30(10): 1129-1137.

Clevers, H. (2016). Modeling Development and Disease with Organoids. Cell, 165(7): 1586-1597.

Heydari, Z., Moeinvaziri, F., Agarwal, T., Pooyan, P., Shpichka, A., Maiti, T. K., Timashev, P., Baharvand, H., & Vosough, M. (2021). Organoids: a novel modality in disease modeling. Bio-Design and Manufacturing, 4(4): 689-716.

Kim, J., Koo, B.-K., & Knoblich, J. A. (2020). Human organoids: model systems for human biology and medicine. Nature Reviews Molecular Cell Biology, 21(10): 571-584.

Zhu, Y., Sun, L., Wu, X., Gu, Z., & Zhao, Y. (2024). Engineered Human Organoids for Biomedical Applications. Advanced Functional Materials, 34(11).

Corrò, C., Novellasdemunt, L., & Li, V. S. W. (2020). A brief history of organoids. American Journal of Physiology-Cell Physiology, 319(1):C151-C165.

Han, Y., Yang, L., Lacko, L. A., & Chen, S. (2022). Human organoid models to study SARS-CoV-2 infection. Nature Methods, 19(4): 418-428.

Rossi, G., Manfrin, A., & Lutolf, M. P. (2018). Progress and potential in organoid research. Nature Reviews Genetics, 19(11): 671-687.

Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., & Jones, J. M. (1998). Embryonic Stem Cell Lines Derived from Human Blastocysts. Science, 282(5391): 1145-1147.

Teshigawara, R., Hirano, K., Nagata, S., Ainscough, J., & Tada, T. (2015). OCT4 Activity during Conversion of Human Intermediately Reprogrammed Stem Cells to iPS Cells through MET. Development, 143(1):15-23.

Tang, X.-Y., Wu, S., Wang, D., Chu, C., Hong, Y., Tao, M., Hu, H., Xu, M., Guo, X., & Liu, Y. (2022). Human organoids in basic research and clinical applications. Signal Transduction and Targeted Therapy, 7(1): 168.

Balistreri, C. R., De Falco, E., Bordin, A., Maslova, O., Koliada, A., & Vaiserman, A. (2020). Stem cell therapy: old challenges and new solutions. Molecular Biology Reports, 47(4): 3117-3131.

Loya, K. (2014). Stem Cells. In Handbook of Pharmacogenomics and Stratified Medicine (pp. 207-231). Elsevier.

Hammond-Browning, N. (2012). Stem Cells. In Encyclopedia of Applied Ethics (pp. 227-234). Elsevier.

Yi, S. A., Zhang, Y., Rathnam, C., Pongkulapa, T., & Lee, K. (2021). Bioengineering Approaches for the Advanced Organoid Research. Advanced Materials, 33(45): 2007949

Zhao, Z., Chen, X., Dowbaj, A. M., Sljukic, A., Bratlie, K., Lin, L., Fong, E. L. S., Balachander, G. M., Chen, Z., Soragni, A., Huch, M., Zeng, Y. A., Wang, Q., & Yu, H. (2022). Organoids. Nature Reviews Methods Primers, 2(1), 94.

Aisenbrey, E. A., & Murphy, W. L. (2020). Synthetic alternatives to Matrigel. Nature Reviews Materials, 5(7): 539-551.

Murphy, S. V, & Atala, A. (2014). 3D bioprinting of tissues and organs. Nature Biotechnology, 32(8): 773-785.

Brassard, J. A., Nikolaev, M., Hübscher, T., Hofer, M., & Lutolf, M. P. (2021). Recapitulating macro-scale tissue self-organization through organoid bioprinting. Nature Materials, 20(1): 22-29.

van de Wetering, M., Francies, H. E., Francis, J. M., Bounova, G., Iorio, F., Pronk, A., van Houdt, W., van Gorp, J., Taylor-Weiner, A., Kester, L., McLaren-Douglas, A., Blokker, J., Jaksani, S., Bartfeld, S., Volckman, R., van Sluis, P., Li, V. S. W., Seepo, S., Sekhar Pedamallu, C., … Clevers, H. (2015). Prospective Derivation of a Living Organoid Biobank of Colorectal Cancer Patients. Cell, 161(4): 933-945.

Lancaster, M. A., & Huch, M. (2019). Disease modelling in human organoids. Disease Models & Mechanisms, 12(7):dmm039347

Yang, S., Hu, H., Kung, H., Zou, R., Dai, Y., Hu, Y., Wang, T., Lv, T., Yu, J., & Li, F. (2023). Organoids: The current status and biomedical applications. MedComm, 4(3):e274.

Caipa Garcia, A. L., Arlt, V. M., & Phillips, D. H. (2022). Organoids for toxicology and genetic toxicology: applications with drugs and prospects for environmental carcinogenesis. Mutagenesis, 37(2),:143-154.

Shi S, Verstegen MMA, Roest HP, Ardisasmita AI, Cao W, Roos FJM, de Ruiter PE, Niemeijer M, Pan Q, Ijzermans JNM, & van der Laan LJW (2022). Recapitulating cholangiopathy-associated necroptotic cell death in vitro using human cholangiocyte organoids. Cell Mol Gastroenterol Hepatol.,13(2):541-564.

Gu S, Wu G, Lu D, Meng G, Wang Y, Tang L, & Zhang W. (2023). Nephrotoxicity assessment of Esculentoside A using human-induced pluripotent stem cell-derived organoids. Phytother Res., 30(10): 4893-4903.

Xu, H., Lyu, X., Yi, M., Zhao, W., Song, Y., & Wu, K. (2018). Organoid technology and applications in cancer research. Journal of Hematology & Oncology, 11(1):116

Fan, H., Demirci, U., & Chen, P. (2019). Emerging organoid models: leaping forward in cancer research. Journal of Hematology & Oncology, 12(1): 142.

Lo, Y.-H., Karlsson, K., & Kuo, C. J. (2020). Applications of organoids for cancer biology and precision medicine. Nature Cancer, 1(8):761-773.

Xu, X., Cheung, S., Jia, X., Fan, G., Ai, Y., Zhang, Y., & Liang, Q. (2024). Trends in organ-on-a-chip for pharmacological analysis. TrAC Trends in Analytical Chemistry, 180, 117905.

Whitesides, G. M. (2006). The origins and the future of microfluidics. Nature, 442(7101):368-373.

Driver, R., & Mishra, S. (2023). Organ-On-A-Chip Technology: An In-depth Review of Recent Advancements and Future of Whole Body-on-chip. BioChip Journal, 17(1):1-23.

Park, S. E., Georgescu, A., & Huh, D. (2019). Organoids-on-a-chip. Science, 364(6444): 960-965.

Wang, H., Ning, X., Zhao, F., Zhao, H., & Li, D. (2024). Human organoids-on-chips for biomedical research and applications. Theranostics, 14(2): 788-818.

Joseph, X., Akhil, V., Arathi, A., & Mohanan, PV. (2022). Comprehensive Development in Organ-On-A-Chip Technology. Journal of Pharmaceutical Sciences, 111(1):18-31.

Zhai, J., Yi, S., Jia, Y., Mak, P.-I., & Martins, R. P. (2019). Cell-based drug screening on microfluidics. TrAC Trends in Analytical Chemistry, 117, 231-241.

Wang, Y., Gao, Y., Pan, Y., Zhou, D., Liu, Y., Yin, Y., Yang, J., Wang, Y., & Song, Y. (2023). Emerging trends in organ-on-a-chip systems for drug screening. Acta Pharmaceutica Sinica B, 13(6): 2483-2509.

Liu, X., Zheng, W., & Jiang, X. (2019). Cell-Based Assays on Microfluidics for Drug Screening. ACS Sensors, 4(6): 1465-1475.

Toh, Y.C., Lim, T.C., Tai, D., Xiao, G., van Noort, D., & Yu, H. A. (2009) microfluidic 3D hepatocyte chip for drug toxicity testing. Lab Chip, 9, 2026-2035.

Li, Z., Hui, J., Yang, P., & Mao, H. (2022). Microfluidic Organ-on-a-Chip System for Disease Modeling and Drug Development. Biosensors, 12(6): 370.

Sontheimer-Phelps, A., Hassell, B. A., & Ingber, D. E. (2019). Modelling cancer in microfluidic human organs-on-chips. Nature Reviews Cancer, 19(2): 65-81.

Liu, X., Fang, J., Huang, S., Wu, X., Xie, X., Wang, J., Liu, F., Zhang, M., Peng, Z., & Hu, N. (2021). Tumor-on-a-chip: from bioinspired design to biomedical application. Microsystems & Nanoengineering, 7(1), 50.

Hassell BA., Goyal G., Lee E., Sontheimer-Phelps A., Levy O., Chen CS., & Ingber DE. (2017). Human Organ Chip Models Recapitulate Orthotopic Lung Cancer Growth, Therapeutic Responses, and Tumor Dormancy In Vitro. Cell Reports, 21(2): 508-516.

Li, C., Holman, J. B., Shi, Z., Qiu, B., & Ding, W. (2023). On-chip modeling of tumor evolution: Advances, challenges and opportunities. Materials Today Bio, 21, 100724.

Nejati, B., Shahhosseini, R., Hajiabbasi, M., Ardabili, N. S., Baktash, K. B., Alivirdiloo, V., Moradi, S., Rad, M. F., Rahimi, F., Farani, M. R., Ghazi, F., Mobed, A., & Alipourfard, I. (2025). Cancer-on-chip: a breakthrough organ-on-a-chip technology in cancer cell modeling. Medical & Biological Engineering & Computing, 63(2):321-337.

Firatligil-Yildirir, B., Yalcin-Ozuysal, O., & Nonappa. (2023). Recent advances in lab-on-a-chip systems for breast cancer metastasis research. Nanoscale Advances, 5(9): 2375-2393.

Subia, B., Dahiya, U. R., Mishra, S., Ayache, J., Casquillas, G. V., Caballero, D., Reis, R. L., & Kundu, S. C. (2021). Breast tumor-on-chip models: From disease modeling to personalized drug screening. Journal of Controlled Release, 331, 103-120.

Moccia C., & Haase K. (2021). Engineering Breast Cancer On-chip-Moving Toward Subtype Specific Models. Front. Bioeng., 9, 694218.

Silvani, G., Basirun, C., Wu, H., Mehner, C., Poole, K., Bradbury, P., & Chou, J. (2021). A 3D-Bioprinted Vascularized Glioblastoma-on-a-Chip for Studying the Impact of Simulated Microgravity as a Novel Pre-Clinical Approach in Brain Tumor Therapy. Advanced Therapeutics, 4(11):2100106

Yi, H.-G., Jeong, Y. H., Kim, Y., Choi, Y.-J., Moon, H. E., Park, S. H., Kang, K. S., Bae, M., Jang, J., Youn, H., Paek, S. H., & Cho, D.-W. (2019). A bioprinted human-glioblastoma-on-a-chip for the identification of patient-specific responses to chemoradiotherapy. Nature Biomedical Engineering, 3(7):509-519.