Organ-on-Chip: The Advancing Microfluidic Technology for Theranostics

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ABSTRACT

Organ-on-chip (OoC) technology represents a revolutionary advancement in *in-vitro* modeling by closely mimicking the physiological and structural characteristics of human organs. It offers a significant improvement over existing microfluidic technology by integrating cell culture within these systems. This integration allows OoCs to simulate organ-level functions on a miniaturized platform, where a microenvironment resembling that within tissue is created through the dynamic flow of fluids and interactions between different cell types. This has valuable applications in drug discovery, disease modeling, and personalized medicine. This review delves into the engineering principles behind OoC systems, with an emphasis on their operating principles, construction, key materials, and various theranostic applications, supplemented by case studies. Key examples, such as lung-on-chip, liver-on-chip, and multi-organ systems, are discussed to illustrate OoC's potential in theranostics, particularly in drug testing and disease progression studies. While OoC models provide significant improvements over traditional *in-vitro* methods, their potential to replace preclinical animal testing is still under investigation. Despite its promise, OoC technology faces several challenges, including integration with sensors, reproducibility, scalability, long-term stability, and regulatory hurdles. This review also explores future trends and technological advancements needed for OoC technology to become a standard tool in biomedical research and clinical applications.

Keywords: Organ-on-chip (OoC), Microfluidics, Disease modeling, Lung-on-chip, Drug discovery

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Introduction

Organ-on-chip (OoC) technology represents a groundbreaking advancement in *in-vitro* testing, offering a highly sophisticated system that closely mimics the physiological functions of living organisms without the need for animal or human subjects.[1] This novel engineering has the potential to significantly transform preclinical research by providing more accurate and dynamic models for studying biological responses in a controlled environment. This technology integrates cutting-edge microfluidics with advanced tissue engineering principles to develop a miniaturized *in vitro* platform that closely replicates both the structural and functional characteristics of human organs by mimicking the complex architecture and physiological responses of real tissues, which previously relied on animal models.[2] These devices are small, often the size of a microscope slide, yet powerful in mimicking key organ-level functions, making them an essential tool for drug discovery,[3] disease modeling,[4] and personalized medicine.[2] The core working principle of OoC technology is microfluidics, which involves channeling and manipulating small volumes of fluids at the microscale through predefined paths, allowing for precise and controlled interactions with the substance of interest.

Nevertheless, what makes these systems truly remarkable is their ability to host various cell types and biomolecules, thereby providing a platform that resembles the actual microenvironment inside an organ. This feature enables precise control over the cellular microenvironment, allowing for the recreation of organ-specific conditions such as fluid flow, shear stress, and oxygen gradients.[5] By imitating natural blood flow, microfluidic systems provide a unique way to study tissue responses in a controlled environment that closely resembles real-life (*in vivo*) conditions.[6] This capability has laid the foundation for creating more physiologically relevant models by increasing the number of interactions between cells and substances of interest, surpassing traditional 2D and static 3D cultures, which are mostly limited to fewer biological interactions, and significantly reducing the reliance on animal models.[7] As illustrated in Figure 1, numerous OoC models have been developed to investigate the distinct properties of various tissues.

A notable example of the impact of OoC technology in theranostics—the fusion of therapeutic and diagnostic applications—is its role in drug screening and disease modeling. For instance, a study by Dongeun Huh et al utilized a lung-on-a-chip model to investigate the toxicity of IL-2-induced pulmonary edema in cancer patients. The model demonstrated that IL-2, in conjunction with respiratory movements, increased lung tissue permeability. However, this effect could be mitigated through the co-administration of Angiopoietin-1. Additionally, the model was used to evaluate TRPV4 inhibitors, which showed potential for reducing fluid leakage, offering a promising avenue for developing treatments for pulmonary edema.[9] In another study focusing on liver disease diagnosis, Gori et al developed a liver-on-a-chip device incorporating HepG2 cells—a human liver cancer cell line—cultured under conditions of free fatty acid overload. The microfluidic design replicated the hepatic sinusoid, facilitating nutrient diffusion and waste removal, thus creating a more physiological environment compared to traditional 2D static cultures. This system was tested for parameters such as intracellular lipid accumulation, cytotoxicity, and ROS generation. The liver-on-a-chip model demonstrated gradual lipid buildup and enhanced cell viability, mirroring the

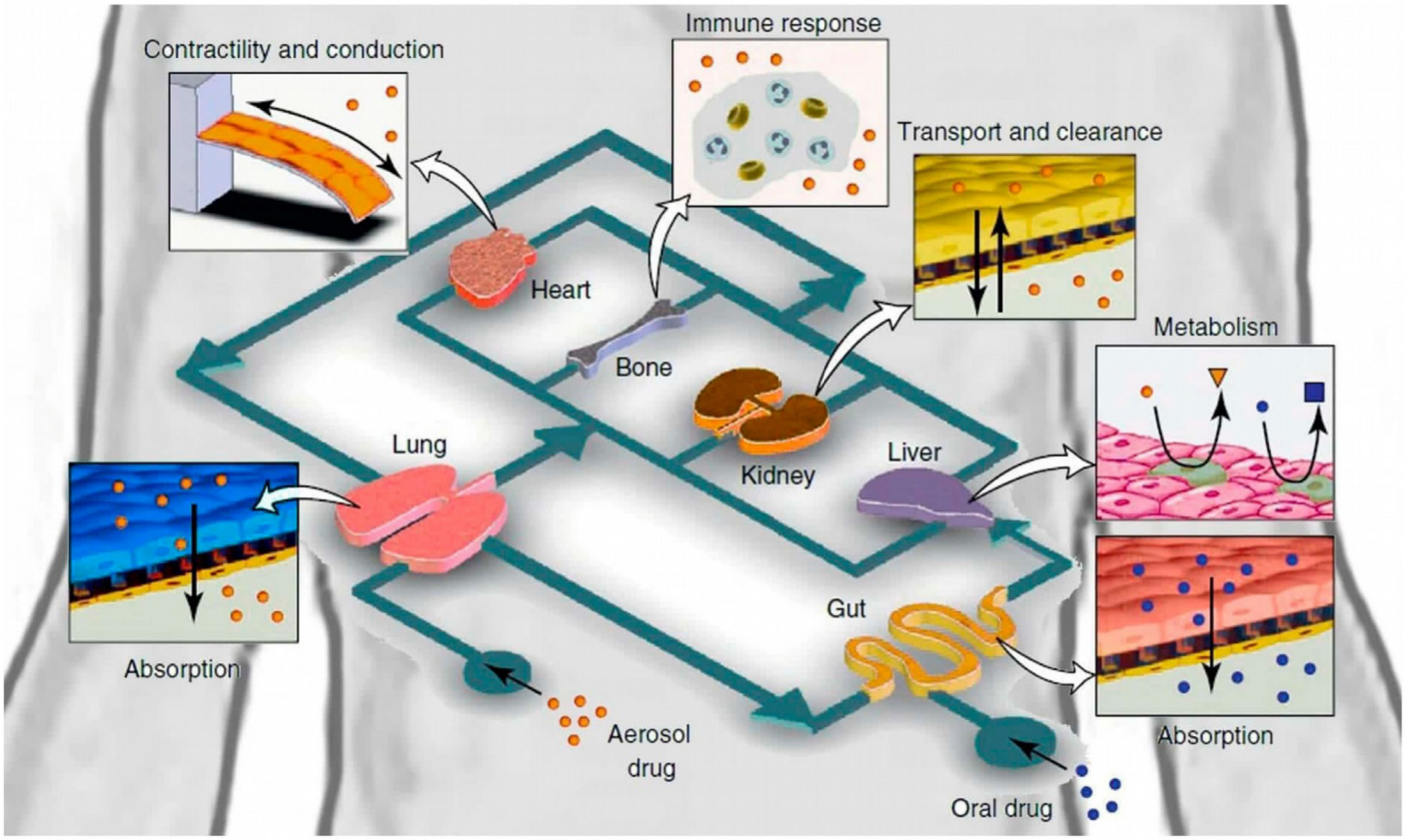

Fig 1 | Various OoC models have been developed for research purposes, each designed to study specific functions. Reproduced from Negar et al, 2024, under CC BY 4.0[8]

chronic progression of non-alcoholic fatty liver disease observed in vivo. Furthermore, the device exhibited potential for long-term culture with minimal oxidative stress, positioning it as a valuable tool for studying liver diseases while reducing dependence on animal models.[10]

Multi-organ systems, often referred to as "body-on-chip" platforms, provide a more in vivo-like environment using human cells, offering the closest simulation of human physiology achievable in a laboratory setting. These interconnected devices replicate the interactions between various organs, facilitating the study of complex diseases and the systemic effects of drugs.[11] Figure 2 shows various multi-organ-on-chip (MOC) combinations that are currently being studied. A notable example is a study by Ronaldson-Bouchard et al,[12] which introduced an advanced multi-organ chip that connects bioengineered heart, liver, bone, and skin tissues via a recirculating vascular flow. The tissues, derived from human induced pluripotent stem cells (hiPSCs), were individually matured for 4–6 weeks prior to integration. The heart tissue underwent electromechanical stimulation; liver tissue maintained metabolic activity; bone tissue exhibited osteoblastic and osteoclastic functions; and skin tissue established barrier integrity. The system's design incorporated a selectively permeable endothelial barrier that preserved tissue-specific environments while allowing communication through immune cells, cytokines, and extracellular vesicles. This modular platform offers flexibility in experimental design by enabling the addition or removal of individual tissues. One of the primary applications demonstrated in the study was drug testing, specifically through the evaluation of doxorubicin, a chemotherapeutic agent known for its cardiotoxic effects. The platform allowed for real-time monitoring of drug effects across multiple tissues, identifying early miRNA biomarkers of cardiotoxicity. Additionally, it simulated tissue injury and the resulting immune response, further mimicking human physiological behavior. This model holds significant promise for personalized medicine, as it can create patient-specific tissue models from hiPSCs, enabling individualized drug testing and disease modeling. MOC systems represent a major step forward in developing more accurate *in vitro* models of human physiology, disease progression, and therapeutic interventions.[12]

This review provides a comprehensive analysis of the engineering principles underlying OoC systems, emphasizing advancements in microfluidic technology that allow these platforms to replicate human organ functions closely. Key applications of OoC technology, including disease modeling, drug discovery, and toxicology testing, will be explored, with case studies illustrating its potential impact on theranostics. Although this technology shows significant promise, it

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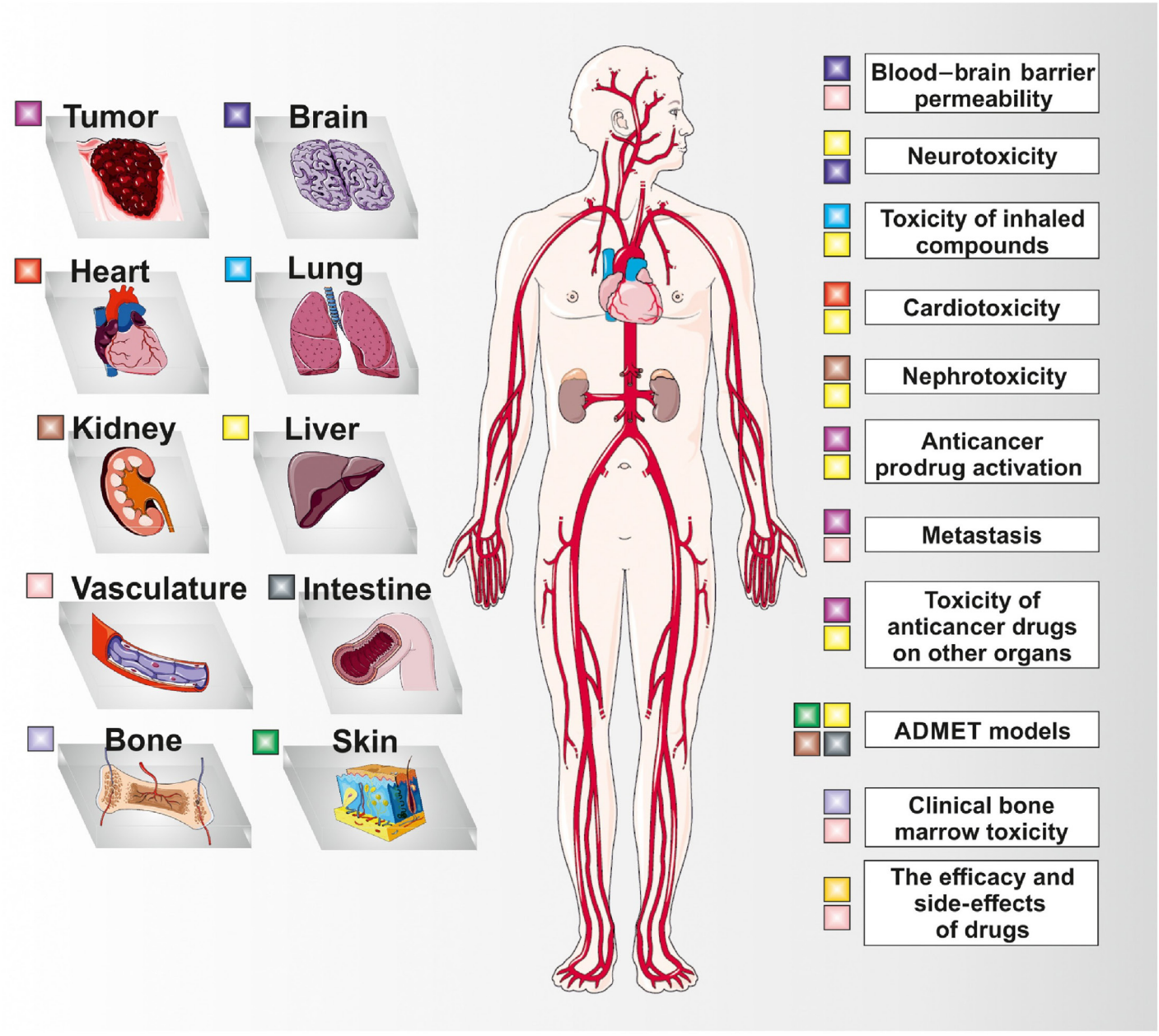

Fig 2 | Various multi-organ-on-chip systems currently under investigation for therapeutic and diagnostic research. Reproduced from Nathalie et al; 2021 under CC BY 4.0[11]

is still in its early stages and cannot yet be considered the ultimate solution for *in vitro* systems. A dedicated section will also address the challenges hindering its widespread adoption and offer insights into the future development of OoC technology.

Engineering Aspects of Organ-on-Chip Technology

Working Principle of OoC

OoCs are specifically engineered to replicate even the most complex physiological functions of human organs or tissues in a controlled laboratory environment. This advanced technology enables researchers to study the functionality or disease modeling of entire organs or tissues, providing valuable insights into their behavior under various conditions.[4] These small, bioengineered platforms are designed to simulate the dynamic structural and functional properties of organs by integrating cellular biology, engineering, and microfluidics. The central principle of an OoC involves creating a microenvironment that closely mimics the cellular and mechanical characteristics of specific organs.[1,3] This is achieved by seeding living cells—such as epithelial, endothelial, or stromal cells—onto a chip made from flexible, transparent materials like polydimethylsiloxane (PDMS). The chip is typically equipped with microchannels and chambers that facilitate fluid flow, akin

to blood circulation, supplying the cells with nutrients. This fluid flow mimics key physiological conditions, such as shear stress, tissue deformation, and chemical gradients, allowing the cells to behave in a manner similar to that in a natural organ.[2]

Mathematical modeling is instrumental in optimizing the design and functionality of these systems. Fluid dynamics models, including the Navier-Stokes equations, simulate blood flow through the microchannels, while diffusion models based on Fick's law predict the transport of gases and nutrients.[13] Additionally, tissue mechanics models are used to simulate the mechanical stretching or contraction of tissues, such as stretching or pulsatile pressure, particularly relevant for organs such as the heart or lungs. Shear flow is applied through laminar, pulsatile, or interstitial flow within microchannels, designed to mimic blood and other fluid flows experienced by tissues. This model is essential for studying cellular responses to varying flow conditions. Next, compression forces are implemented using devices like pistons or diaphragms to apply pressure on cells, simulating conditions found in bone or cardiac tissues where compressive stress is common. Finally, stretch or strain is achieved by cyclically applying vacuum pressure, which recreates the stretching forces experienced by tissues such as lung cells during breathing. These models together enable OOC platforms to replicate realistic cellular environments, facilitating controlled studies of cellular responses to physical forces and enhancing the relevance of *in vitro* tissue models.[14] For example, a lung-on-chip may incorporate membranes that stretch to simulate breathing, while a heart-on-chip could replicate contractions.[15] Cellular interaction models and pharmacokinetics/pharmacodynamics models further enhance the biological relevance of these systems by predicting cellular responses, drug interactions, and nutrient dynamics. These models shift from traditional PK-PD approaches to physiologically-based PK models, which assess drug distribution across interconnected organ systems. MOCs, leveraging microfluidics and bioengineered tissues, enable close replication of in vivo conditions by connecting organ models like the liver and heart to capture drug interactions. Such models improve the prediction of drug efficacy, toxicity, and metabolism, ultimately enhancing *in vitro*-to-*in vivo* extrapolations for drug testing and disease modeling.[16] OOC technology enables the detailed study of organ-level functions,[17] disease mechanisms,[18] drug responses and toxicity,[16] and reducing reliance on animal models.[20] These systems can integrate sensors to monitor parameters like oxygen levels, fluid flow, and cellular responses in real time.[21] OoC technology, which stems from advancements in microfluidics, offers a unique capability to interact with multiple cell types simultaneously. This makes it an ideal candidate for studying near-human physiological interactions, presenting advantages over conventional 2D or 3D cell cultures. However, despite its potential, significant improvements are still required to achieve the same level of efficacy as in vivo testing.

Construction of OoC's

The fabrication of OoC devices is a multidisciplinary process that blends microfluidics, biomaterials, tissue engineering, and cell biology to create functional microenvironments that mimic human organs.[22] This process involves several key fabrication techniques and material considerations that vary depending on the type of organ being modeled. Typically, the fabrication techniques used in OoC can be divided into three categories: subtractive manufacturing, formative manufacturing, and additive manufacturing.[23]

- ***Subtractive manufacturing:*** This process involves removing material from a solid block to create the desired shape or structure. In the context of OoC devices, this method can be used to fabricate precise microfluidic channels in materials such as silicon, glass, or polymers. For example, a study by Alver et al fabricated the SliceChip platform using a micro milling technique, which supports the long-term culture of pancreatic slices with controlled oxygenation, perfusion, and stability. This enables continuous assessments of insulin secretion, advancing diabetes research and offering the potential for studying other tissue types, including tumors.[24]
- ***Formative manufacturing:*** In this category, materials are shaped using methods such as hot embossing and injection molding, which are highly effective for mass production. In a study by Tiffany et al, the authors fabricated a master mold using Digital Light Processing 3D printing or Stereolithography (SLA) 3D printing. PDMS was then cast into these molds to create a blood-brain barrier chip, used to study cell-to-cell interaction with the application of physiological shear stress, better-mimicking blood flow in the brain. They also created an Airway-On-Chip by culturing a monolayer of epithelial cells on a porous membrane to test the interaction of drugs with lung tissue.[25]
- ***Additive manufacturing:*** Additive techniques, such as 3D printing, are becoming increasingly popular due to their flexibility in creating complex geometries. Extrusion-based and inkjet-based 3D printing allows for the precise deposition of bioinks, facilitating the construction of scaffolds and microenvironments with tailored cellular patterns. A study by Steinberg et al developed a fully 3D-printed tumor-on-a-chip model using SLA 3D printing, which enables multi-drug screening with patient-derived tumor spheroids. The model exhibited biocompatibility and displayed a high degree of alignment between drug responses in lab settings and real patient outcomes, suggesting its potential as a tool for personalized cancer therapy.[26]

The choice of material is a critical aspect of OoC design. The materials used must be biocompatible,

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mechanically robust, and often optically transparent to facilitate real-time imaging and analysis. Common materials used in OoC systems are discussed in Table 1 below.

Design Considerations for Various OoCs

A critical challenge in OoC fabrication is designing systems that accurately replicate the physiological environment of an organ. This involves controlling fluid flow, mechanical stress, and biochemical gradients within the device.[36] For instance, flow systems in microfluidic channels are designed to replicate blood flow or nutrient supply, which is essential in maintaining the viability of cells in systems like blood vessel-on-a-chip and kidney-on-a-chip.[37] MOC devices, like lung-on-a-chip, mimic the physical forces on cells by using cyclic stretching to recreate the lung's expansion and contraction during breathing.[38] Similarly, kidney-on-a-chip systems use shear stress to simulate the filtration dynamics of renal epithelial cells. These forces are key to maintaining cellular functions in an in vivo-like environment.[38] A major advancement in OoC technology is the integration of multiple cell types within a single chip. A design challenge is multi-organ integration, where several OoCs are connected to replicate inter-organ communication. This "body-on-a-chip" approach is especially useful for drug toxicity studies, as it mimics the interaction between different organs, such as the liver and kidney.[39] For systems like pancreas-on-a-chip, which require intricate interactions between different cell types (e.g., insulin-secreting beta cells and glucagon-secreting alpha cells), microfluidic devices can simulate the dynamic glucose regulation in the body. This setup is valuable for studying diseases like diabetes.[40] Similarly, liver-on-a-chip models aim to replicate hepatocyte interactions within a 3D matrix to assess liver functions like detoxification.[10]

Theranostic Applications Using OoCs

Various OoC Models

Several OoC models have been developed over the past few years, particularly since the introduction of the first lung-on-a-chip model by Huh et al in 2007.[41] Today, nearly all human organs have been replicated in microfluidic models to study their complex interactions. Table 2 below provides a comprehensive list of OoCs developed to date.

OoC Models and Case Studies

Several OoC models have been developed over the past two decades, with the most extensively researched models being those for the lungs, liver, and tumors.[80] This focus is primarily due to the essential functions of these organs, their disease prevalence in clinical settings, their diverse roles in drug metabolism, and the high incidence of cancer in these organs, making them critical for therapeutic testing and development.[80,81] The limitations of traditional *in-vitro* models in replicating diseased states have driven the development of these OoC models. However, with the need to accelerate disease modeling and preclinical testing, an increasing number of models are now being explored.[82] With advancements in technology, OoCs are now able to create near-accurate microenvironments for several organs, incorporating dynamic circulation and achieving a degree of actual tissue complexity within the laboratory.[2,83] Below are various engineering approaches demonstrated by research teams worldwide.

In a study presented by Pauline et al, a second-generation lung-on-a-chip model replicates the structure and function of the human lung alveoli more accurately than previous models, as depicted in Figure 3. The primary innovation is the use of a collagen-elastin membrane that mimics the extracellular matrix of lung tissue, replacing synthetic materials like PDMS. The goal was to better recreate the complex mechanical

Table 1 | Various Materials Used in the Construction of OoCs

Type of Material	Material	Features	Ref
Polymers Hydrogels Silicon Based Materials Ceramics	Polydimethylsiloxane	Flexible, transparent, biocompatible, gas-permeable, easy to mold	27
	Polyethylene glycol diacrylate	Hydrophilic and tunable mechanical properties	28
	Polymethyl methacrylate	Rigid, transparent, biocompatible	29
	Polycarbonate	High mechanical strength and optical transparency	29
	Polyurethane	Good elasticity and strength	30
	Collagen	Naturally occurring protein mimics extracellular matrix	22
	Matrigel	Gelatinous protein mixture simulating extracellular matrix	22
	Alginate	Biocompatible and gelforming	22
	Fibrin	Cell adhesion properties	31
	Gelatin Methacrylate	Tunable mechanical properties, photo-crosslinkable	32
	Silicon	High mechanical strength, easy to fabricate	33
	Glass	Optically transparent, non-porous	34
	Zirconia	High mechanical strength, biocompatibility	35

Table 2 | A Comprehensive List of All OoC Models Developed So Far for Research Purposes

Organ System	OoC Model	Functionality	Applications	Ref
Respiratory System	Lung-on-Chip	Mimicsalveolar-capillary interface studies gas exchange, lung diseases, and inhalation toxicity	Drug testing, lung diseases (asthma, COPD), and toxicology	42,43
	Airway-on-Chip	Replicates bronchial/trachea l tissues, studies asthma, COPD, and infections	Asthma, respiratory infections, and lung diseases	44
Cardiovascular System	Heart-on-Chip	Simulates cardiac muscle contraction and electrophysiology	Cardiovascular diseases, drug-induced cardiotoxicity, heart disease research	45
	Blood Vessel-on-Chip	Models endothelial cells in blood vessels	Thrombosis, atherosclerosis, and vascular biology	46,47
	Vascularized Organ-on-Chip	Integrates vascular networks with other organs	Tissue perfusion, multi-organ studies	47
Nervous System	Brain-on-Chip	Mimics neural tissue and circuits, models brain physiology	Alzheimer's, Parkinson's, epilepsy, and neurological diseases	48
	Blood-Brain Barrier (BBB)-on-Chip	Models the selective transport properties of the BBB	Neurotoxicity, drug delivery, and brain diseases	49
	Neurovascular Unit-on-Chip	Combines neurons, glia, and vascular cells for brain-blood interaction	Neurodegenerative diseases, brain research	50
Digestive System	Gut-on-Chip	Emulates intestinal epithelium and microbiota interactions	Digestion, inflammatory bowel disease, drug absorption, microbiota research	51
	Stomach-on-Chip	Mimics gastric tissues and secretions	Digestion, drug absorption, and stomach diseases	52
	Liver-on-Chip	Replicates liver metabolism, detoxification, and protein synthesis	Hepatitis, liver diseases, drug metabolism, and hepatotoxicity testing	10,53
	Pancreas-on-Chip	Models insulin production and glucose regulation	Diabetes research and insulin regulation	54
	Bile Duct-on-Chip	Mimics bile duct tissue	Liver and bile duct diseases, cholestasis	55
	Esophagus-on-Chip	Models esophageal tissue	Acid reflux, esophageal cancer research	56
Renal System	Kidney-on-Chip	Mimics nephron filtration and reabsorption	Kidney diseases, nephritis, diabetic nephropathy, and nephrotoxicity studies	57
Renal System	Glomerulus-on-Chip	Specifically models glomerular filtration barrier	Protein leakage studies, kidney disease	58
	Proximal Tubule-on-Chip	Focuses on toxin and drug transport	Kidney function, nephrotoxicity, and drug testing	59
Musculoskele tal System	Bone-on-Chip	Emulates bone remodeling and regeneration	Osteoporosis, bone cancer, and fracture healing	60
	Cartilage-on-Chip	Mimics cartilage tissue dynamics	Arthritis, joint degeneration, cartilage regeneration	61
	Skeletal muscle-on-Chip	Models skeletal muscle contraction	Muscular dystrophy, muscle regeneration, and injury healing	62
	Tendon-on-Chip	Focuses on tendon function and tissue mechanics	Injury healing and biomechanical studies	63
Endocrine System	Pancreas-on-Chip	Models insulin-secreting cells	Diabetes, glucose regulation, and endocrine research	40
	Thyroid-on-Chip	Replicates thyroid hormone regulation	Thyroid diseases (hyperthyroidism, hypothyroidism)	64
Reproductive System	Uterus-on-Chip	Emulates uterine tissue for implantation and menstruation studies	Endometriosis, menstrual cycle studies, implantation research	65
	Placenta-on-Chip	Mimics maternal-fetal interface	Drug transfer, placental diseases, fetal development	66
	Ovary-on-Chip	Models follicle development and hormone secretion	Reproductive health, fertility research	67
	Testis-on-Chip	Mimics spermatogenesis and hormone production	Male fertility, testicular diseases, and hormone regulation	68
Skin and Sensory Organs	Skin-on-Chip	Replicates skin barrier properties and immune responses	Dermatology research, cosmetic testing, wound healing	69
	Eye-on-Chip	Models retinal and corneal tissues	Eye diseases (glaucoma, cataracts), diabetic retinopathy research	70
	Ear-on-Chip	Mimics cochlear and auditory function	Hearing loss, ototoxicity studies	71
Immune System	Thymus-on-Chip	Simulates T-cell maturation	Thymic diseases, immune development	72
	Spleen-on-Chip	Mimics spleen functions	Splenic diseases, immune response studies	73
	Lymph Node-on-Chip	Models lymphatic tissue	Cancer metastasis, immune response	74
Cancer Models	Prostate Cancer-on-Chip	Models prostate cancer tissues	Prostate cancer progression, drug testing	75,76
	Lung Cancer-on-Chip	Models lung tumor microenvironments	Lung cancer research, tumor progression, drug testing	75,77
	Breast Cancer-on-Chip	Mimics breast tumor tissues	Breast cancer progression, personalized therapies	78
	Pancreatic Cancer-on-Chip	Mimics pancreatic tumor microenvironments	Pancreatic cancer, aggressive cancer research	75,77
	Colorectal Cancer-on-Chip	Models colon tumor environment	Colorectal cancer research, tumorigenesis, drug screening	75,77
Circulatory Models	Blood-on-Chip	Models blood flow and coagulation	Thrombus formation, blood disorders research	79

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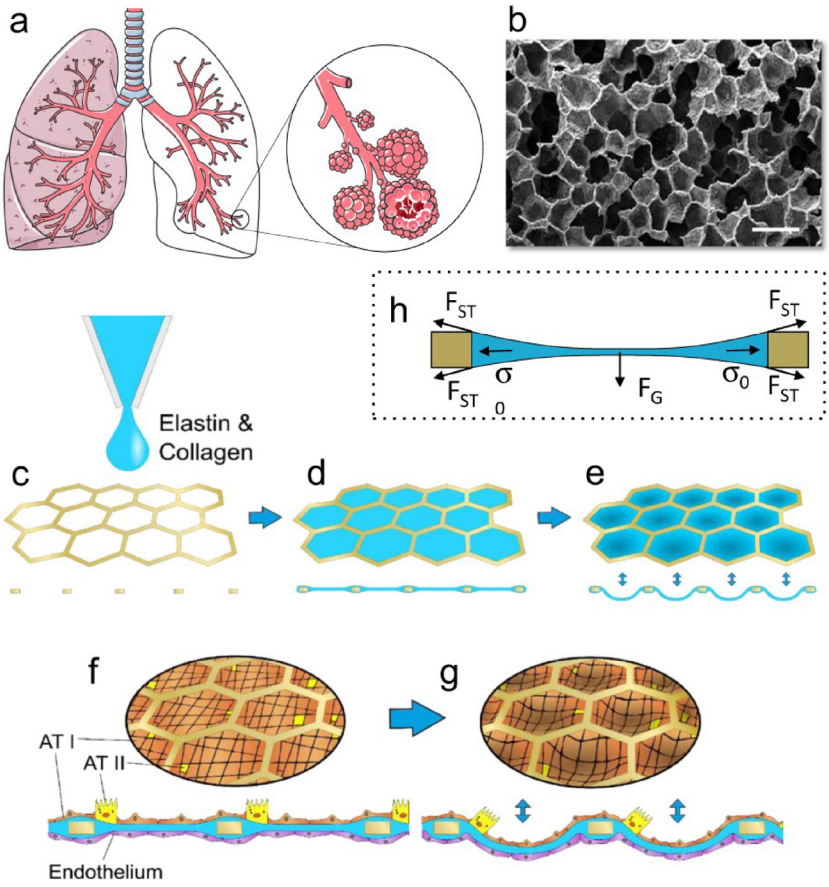

Fig 3 | Lung-on-chip confirmation (a) A detailed schematic of the branching respiratory tree-like structure, progressively dividing into smaller bronchioles, ultimately terminating in clusters of alveolar sacs for gas exchange. (b) Scanning electron microscope (SEM) image of a human lung parenchyma slice, revealing small alveoli and their ultrathin air-blood barrier crucial for efficient gas exchange (Scale bar 500 μm). (c–d) Illustration showing the production of the CE membrane for the second-generation lung-on-a-chip involves using a thin gold mesh scaffold with a hexagonal pore array, each pore approximately 260 μm in size. A drop of collagen-elastin solution is carefully pipetted onto the scaffold, forming a flexible and biomimetic membrane for the device. (e) The collagen-elastin gel creates a delicate, suspended membrane that can be stretched at the alveolar level by applying a controlled negative pressure to the basolateral side, mimicking natural tissue dynamics. (f–g) An illustration showing the alveolar-capillary interface. Primary human lung alveolar epithelial cells, both type I (ATI) and type II (ATII), are cocultured with lung endothelial cells on a thin, flexible collagen-elastin membrane. (h) The force balance during membrane drying involves several factors: FST represents the surface tension force, FG accounts for the influence of gravity, and σo denotes the membrane's inherent residual stress. Reproduced from Pauline et al; 2021 under the CC BY 4.0[84]

and biochemical environment of lung alveoli *in vitro*. The biological membrane, made from collagen and elastin, is created by pipetting a CE solution onto a gold mesh, where it forms a thin, stretchable membrane through surface tension and evaporation. The mesh provides a structural scaffold for an array of 40 alveoli with dimensions similar to those found in vivo. The researchers cultured human lung alveolar epithelial cells and endothelial cells on both sides of the membrane to model the air-blood barrier. They observed that the membrane not only supported cell growth but also allowed for mechanical stretching that mimicked breathing motions, with cells forming a functional barrier over several weeks.[84]

Compared to PDMS membranes, the CE membrane showed superior properties, such as better permeability, optical clarity, biodegradability, and lower absorption of small molecules. The cells maintained their phenotypes, expressed typical alveolar markers, and demonstrated robust tight junction formation, crucial for mimicking the lung's barrier function. Additionally, the stretchable CE membrane showed mechanical resilience under cyclic negative pressure, closely simulating physiological strain during breathing. This second-generation lung-on-a-chip platform addresses several limitations of earlier models by better mimicking the alveolar environment in terms of geometry, mechanical properties, and cell-matrix interactions. This system has potential applications in drug screening, disease modeling, and personalized medicine, providing a more accurate representation of the lung's responses to treatments or pathological conditions.[84]

Another model developed by Kwon et al, as in Figure 4, presents a liver acinus dynamic (LADY) chip designed to model the liver's zonation and assess drug-induced zonal hepatotoxicity. This innovative chip mimics the structure of a liver acinus, recapitulating key features such as zonal expression patterns and metabolic functions of the liver. The LADY chip consists of HepG2 cells cocultured with human umbilical vein endothelial cells (HUVECs) to more accurately reflect the liver's microenvironment. In this study, the chip was developed to simulate the flow of oxygen and nutrients, as seen in vivo, from periportal zone 1 to perivenous zone 3. The result was the formation of liver zonation, with different zones showing varied metabolic activity—zone 1 hepatocytes expressed higher levels of the enzyme phosphoenolpyruvate carboxykinase, while zone 3 hepatocytes showed elevated levels of cytochrome P450 2E1. This zonal distinction is critical in assessing the liver's response to drugs. The researchers tested acetaminophen (APAP)-induced hepatotoxicity and found that zone 3 cells were more susceptible to cell death due to higher metabolic activation of APAP in that region, correlating with the natural function of CYP2E1 in drug metabolism. Interestingly, coculturing HepG2 cells with HUVECs resulted in enhanced resistance to APAP-induced toxicity, likely due to the supportive interactions between hepatocytes and endothelial cells. Overall, the LADY chip successfully mimics liver zonation and offers a promising tool for drug testing, especially in evaluating zonal hepatotoxicity, reducing reliance on traditional animal models that may not fully predict human liver responses. The study also suggests future applications in personalized medicine and the testing of drug candidates for liver toxicity.[85]

Tumor-on-chip models are gaining widespread attention due to their efficacy in mimicking the tumor microenvironment. A great example is a study by Mehta et al.,[86] in which the team introduces a novel microfluidic device for personalized drug testing in oral cancer, as illustrated in Figure 5. This research addresses key challenges in cancer treatment, such as tumor heterogeneity and drug resistance, by

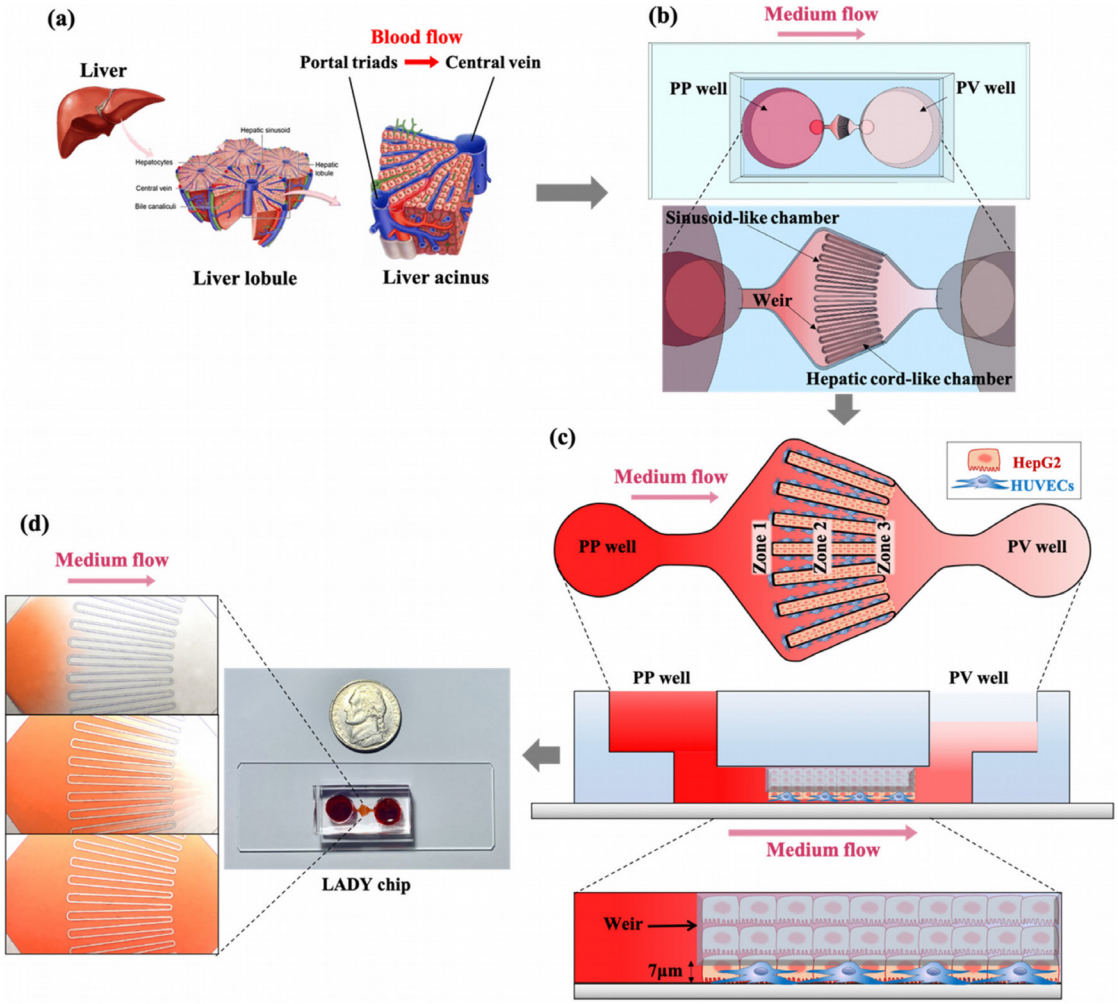

Fig 4 | Illustration of the working principle of a liver acinus dynamic (LADY) chip. (a) The LADY chip was developed based on the complex architecture and specialized functions found in the liver acinus, which is part of the lobule. By mimicking key aspects of the acinus microenvironment, the chip aims to provide a more accurate model of liver tissue for studying cellular behavior and liver-specific processes. (b) This figure presents a top-down view and detailed microstructure of the LADY chip. The hepatic cord-like cell chambers are organized in a radial pattern, alternating with chambers that resemble sinusoids, reflecting the natural architecture of liver tissue. (c) This figure illustrates the coculture of HepG2 cells and HUVECs within the LADY chip. HUVECs were introduced into the periportal (PP) well, while HepG2 cells were added to the perivenous (PV) well, where both cell types were captured by a weir structure positioned 7 μm above the chip's base in a partial radial layout, mimicking the liver acinus microstructure. The design, aligned with liver acinus functionality and blood flow direction, facilitated the establishment of metabolic zonation from zone 1 to zone 3, as the medium flowed from the PP well to the PV well through the fenestrated weir. (d) An actual photograph of with LADY chip with red dye-filled microchambers. Reproduced from Kwon et al;2022 under CC BY 4.0[85]

developing a dynamic, patient-derived spheroid model for testing drug combinations. The device was designed to overcome the limitations of previous models, including lengthy fabrication, absence of cancer stem cells, and lack of clinical correlation. The major thrust of this study includes the development of a 3D-printed, mold-based microfluidic platform with serpentine loops that allow for the mixing and testing of seven combinations of three chemotherapeutic drugs (paclitaxel, 5-fluorouracil, and cisplatin). Patient-derived cancer stem-like spheroids exhibited significant differences in drug responses, correlating with tumor differentiation and clinical diagnoses. For instance, spheroids from patients with well-differentiated tumors responded differently compared to those from moderately differentiated tumors. The study also revealed varying drug resistance profiles, with patient 1's spheroids showing the highest resistance, which was associated with hypoxia and reduced cell proliferation. Another key finding was the successful maintenance of patient-derived spheroids under clinically relevant oxygen levels (below 5% O_2), replicating the tumor microenvironment more accurately. This study demonstrated that tumor differentiation status significantly influences drug response, which has rarely been explored in prior research. The research also highlighted the importance of hypoxia in contributing to drug resistance, particularly in patient 1's

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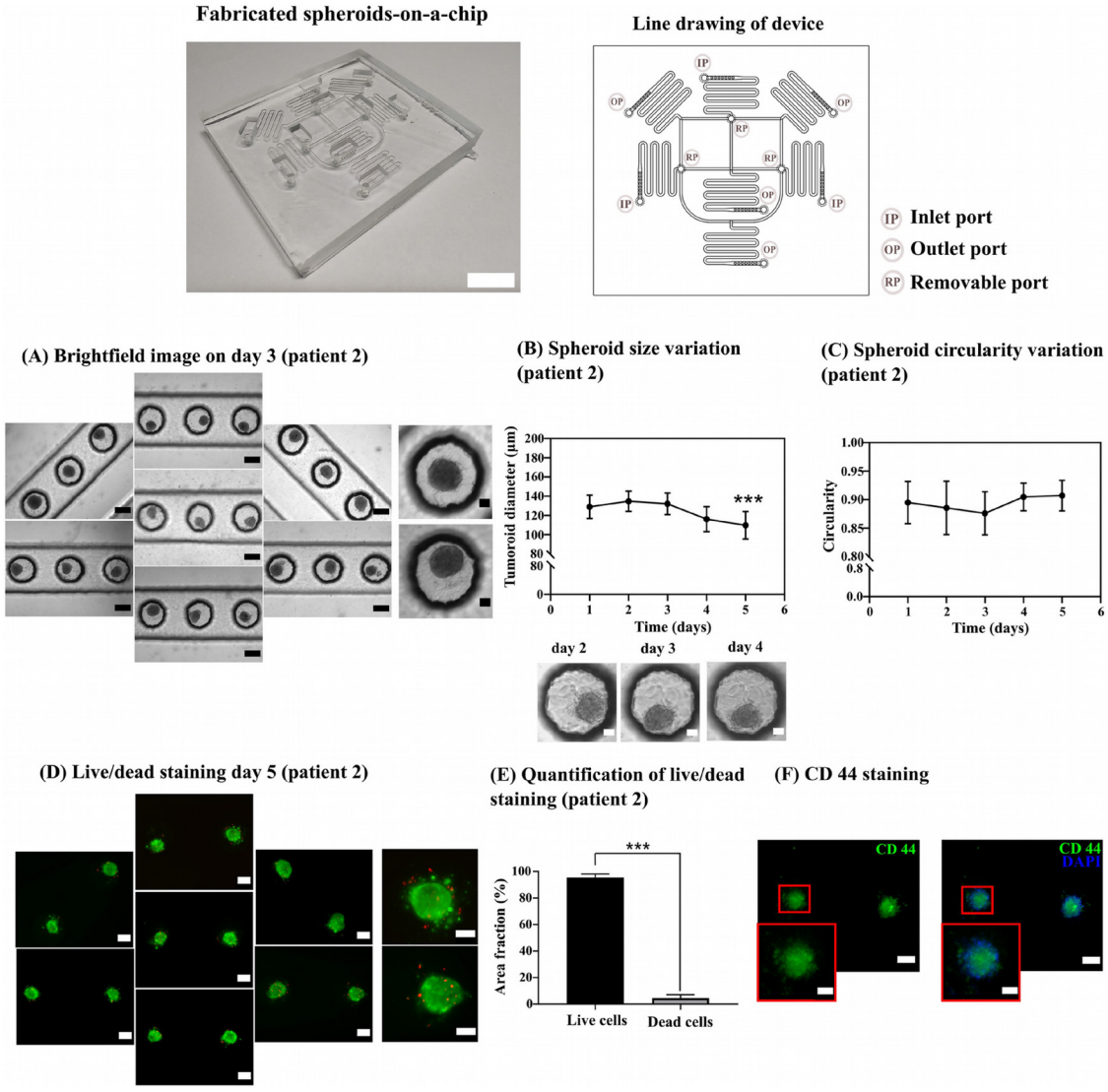

Fig 5 | Photograph and schematics with major observations of the tumor-on-chip model. (a) Primary oral stem-like tumor cells from patient two were seeded at a flow rate of 12 µl/min. Brightfield microscopy images of the spheroids were captured on day 3, where the left panel includes a scale bar of 200 µm, and the magnified right panel features a scale bar of 50 µm. (b) The change in spheroid size over time was assessed using ImageJ software (scale bar: 50 µm), with the data expressed as mean ± SD. Statistical analysis using a Student's t-test showed a significant difference when compared to day 1 ($p < .001$, (***), n = 49 spheroids). (c) The circularity of the spheroids over time was evaluated using ImageJ, with results reported as mean ± SD (n = 49 spheroids). (d) On day 5, live/dead cell staining revealed live cells in green and dead cells in red. The left panel includes a scale bar of 100 µm, while the magnified right panel has a scale bar of 50 µm. (e) Live/dead staining performed on day 5 showed green-stained live cells and red-stained dead cells, with a scale bar of 100 µm in the left panel and 50 µm in the zoomed-in right panel. (f) Immunofluorescence staining using the CD44 marker revealed CD44-positive cells in green, while DAPI-stained nuclei appeared blue (scale bar: 100 µm for the overall view and 50 µm for the zoomed-in view). The spheroids within the microwell arrays are arranged horizontally, displaying the arrays on both the far left and far right sides. Reproduced from Mehta et al; under CC BY 4.0.[86]

spheroids, which exhibited both high hypoxia levels and strong resistance to chemotherapy.[86]

Challenges and Limitations

OoC technology has advanced rapidly, offering novel ways to replicate human organ function and aiding drug discovery. However, despite its potential, this technology faces significant challenges and limitations that must be addressed to realize its full promise. Based on a comprehensive review of multiple sources, the key issues are as follows:

1. Material and manufacturing limitations
   The materials commonly used in OoC technology, such as PDMS, present several problems. PDMS, though popular due to its biocompatibility and ease of fabrication, has limitations, including poor chemical stability and the tendency to

absorb small molecules, which can affect experimental accuracy. Furthermore, manufacturing these chips requires sophisticated microfabrication techniques that can be expensive and time-consuming, making large-scale production a challenge.[87,88]

2. Complexity in mimicking human physiology
While OoCs can replicate the microenvironment of organs, they are still far from capturing the complete complexity of human physiology. For instance, many systems fail to reproduce multi-organ interactions accurately, which is critical for understanding systemic effects such as drug metabolism and immune responses.[88,89] The development of MOC systems has sought to address this, but these integrated systems are still in their infancy and present further engineering challenges, such as the need for universal media that can support multiple cell types.[83]
3. Scalability and standardization issues
OoCs have shown promise in small-scale laboratory settings, but scaling up for widespread commercial use and integration into drug discovery pipelines remains a significant hurdle. There is no standardized protocol for fabricating or operating these devices, and variations in chip design, cell sourcing, and culture conditions can lead to inconsistent results.[88,89] Moreover, the need for specialized equipment to control microfluidic flows and maintain the cells' environment adds further complexity and cost.[87]
4. Integration of immune system components
Another challenge is the difficulty in integrating immune system components into OoC models. Many organ systems interact closely with immune cells, yet incorporating these interactions in a controlled and reproducible manner has proven challenging.[83,90] This is a critical limitation, especially when studying diseases that involve immune responses or testing drugs that affect the immune system.
5. Lack of long-term stability
One of the key advantages of OoCs is the ability to maintain cells in a more physiologically relevant state compared to traditional 2D cultures. However, many OoC platforms struggle to maintain cell viability and function over long periods, limiting their utility for studying chronic diseases or long-term.
6. Ethical and regulatory hurdles
Although OoCs hold the potential to reduce reliance on animal models, ethical and regulatory challenges remain. For instance, the use of induced pluripotent stem cells (iPSCs) to populate chips raises questions about the ownership and commercialization of human genetic material. Moreover, regulatory agencies like the FDA have yet to establish clear guidelines for validating OoC models as reliable tools for drug testing.[88,91]
7. Technical challenges in fluid dynamics
OoCs rely on microfluidics to stimulate blood flow and nutrient transport. However, achieving precise control of fluid dynamics, such as mimicking the pulsatile nature of blood flow or reproducing shear stresses found in certain tissues, remains a technical challenge. The small scale of these systems can lead to surface effects dominating over volume effects, which can skew experimental results.[87,92]
8. Cost and accessibility
Despite the growing interest in OoC technology, the high cost of development and the requirement for specialized technical knowledge limit its accessibility to only well-funded research institutions and large pharmaceutical companies. Reducing the cost and complexity of these systems is essential for broader adoption.[87–89]

Conclusion and Future Trends

In conclusion, OoC technology has demonstrated significant potential in revolutionizing biomedical research, drug development, and personalized medicine.[17] These microfluidic devices replicate human organ structures and physiological processes, providing a more accurate model for drug testing, disease modeling, and toxicity assessments compared to traditional 2D cell cultures or animal models.[37] The integration of multiple organs on a chip and the development of personalized OoCs are key advancements in this field, offering better simulation of human organ interactions and individualized responses to treatments.[93]

Future trends in OoC technology are promising, with ongoing research focusing on multi-organ platforms and the integration of artificial intelligence to analyze complex datasets generated by these chips. Machine learning algorithms can help predict drug responses more efficiently, potentially reducing the need for animal testing and accelerating drug discovery.[94,95] Furthermore, the incorporation of stem cells, including patient-derived iPSCs, into OoC devices can lead to more personalized medicine approaches, where treatments are tailored to the individual's genetic makeup.[96] However, challenges remain, such as the standardization of manufacturing processes and addressing the technical limitations of microfluidic systems, such as fluid dynamics control and material biocompatibility.[92] Addressing these challenges will be crucial for the future development and widespread adoption of OoC technology in both research and clinical settings. Overall, OoCs represent a frontier in biomedical engineering, with the potential to transform personalized medicine and improve the precision and reliability of preclinical testing.

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