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A Comprehensive Review of Organ-on-Chip/Body-on-Chip Systems: Engineering, Applications, and Potential Impact on Drug Development and Administration

Nanxi Jing

University College London, London, WC1E 6BT, United Kingdom

Abstract:

The current drug development process faces significant challenges of a high failure rate that results in substantial time and financial waste. This is primarily attributed to the lack of pre-clinical models capable of generating physiologically relevant data essential for accurate predictions during decision-making for advancing to costly clinical stages. Fortunately, advancements in cell culture technology and material fabrication techniques have given rise to an interdisciplinary innovation known as organ-on-chip/body-on-chip (OoC/BoC), offering unparalleled physiological relevance. This review aims to provide a comprehensive overview of the materials and techniques involved in engineering OoC/BoC systems, covering the sourcing of cells from diverse origins, tissue model creation, material processing for culturing these models and coupling single OoCs into complex BoC systems. Furthermore, the potential applications of OoCs/BoCs in drug discovery processes and personalized medicine are explored. Lastly, we discuss the significant potential of this technology to revolutionize the entire drug development pipeline and the way of drug administration, as well as address the key regulatory obstacles impeding the large-scale application of the technology. In summary, this review underlines the pivotal role of OoC/BoC technology in addressing current limitations in drug development, offering promising avenues for improving efficiency, reducing costs, and advancing personalized medicine.

Keywords: Organ-on-chip; Body-on-chip; Drug screening; Personalized Medicine;3D Bioprinting

Introduction

Within the past decades, the advancement of genetic and proteomic sequencing has revealed a tremendous number of pathological pathways of a variety of diseases, which, therefore, unmasked lists of potential target sites for therapeutic development. Unfortunately, the process of developing corresponding drugs is comparably inefficient. Typically, it would cost over 2 billion dollars and take, on average, 10-15 years for a drug to hit the market[1]. During the drug development pipeline, which is illustrated in Figure 1, 90% of the selected candidates fail at the clinical stages due to the complex interactions of human bodies, which can be financially and temporally devastating. One approach to minimizing such waste is to endeavor to predict the majority number of the interactions at the pre-clinical stage by establishing a system that mimics human microenvironments in vitro.

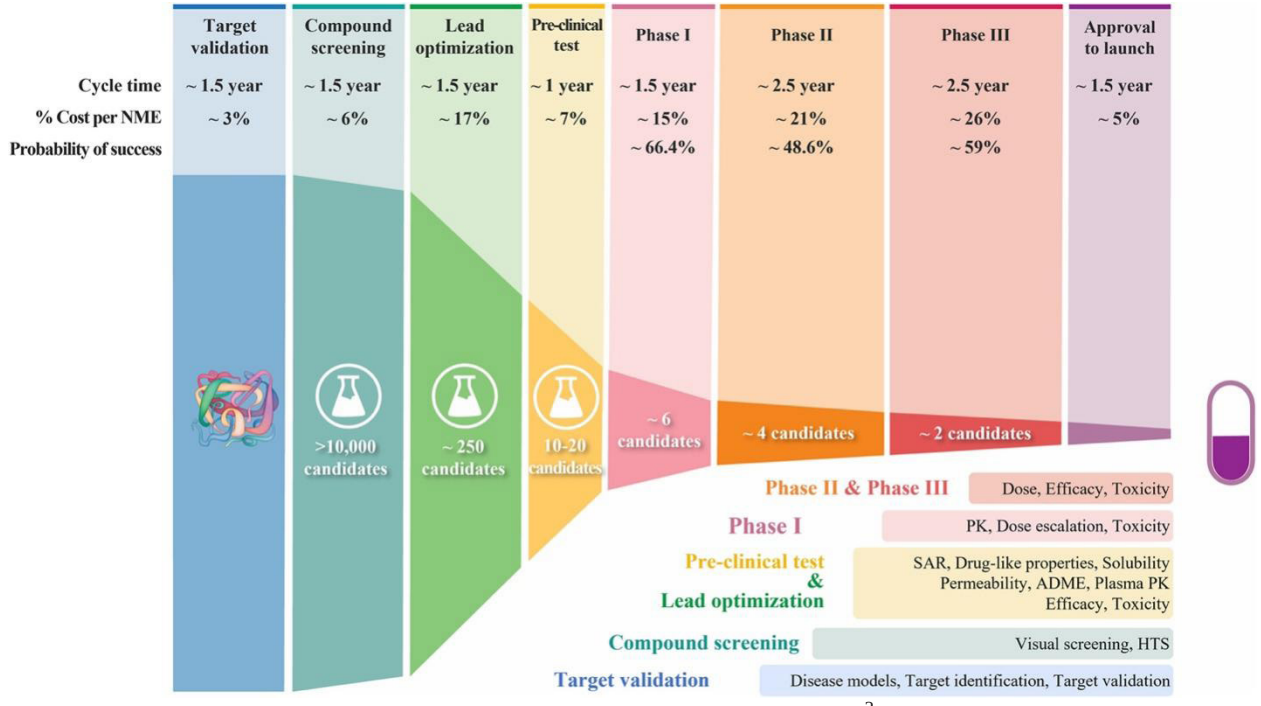

Figure 1. Drug development pipeline[2]

The pre-clinical stages mainly aim to depict the toxicity, efficacy, pharmacodynamics (PD), and pharmacokinetics (PK) of the tested compounds, forecasting their potential behaviors within the human system in the later clinical stages[3]. The first two investigate the effects, both unexpected and desired, of the drug candidates on single organs. In contrast, PD and PK investigations delve into the broader interactions of the drug with the entire body. Specifically, PD explores the physiological and biochemical effects of drugs and how they correlate with drug concentrations over time. In contrast, PK focuses on simulating the drug absorption, distribution, metabolism, and elimination (ADME) process[4]. To understand the intricate process of drug-body interactions, physiological-based mathematical models have been developed, encompassing physiological parameters such as organ sizes, blood flow rates, tissue compositions, and metabolic processes[5]. The obtained information can aid in decision-making on dosing strategies and support the selection of promising drug candidates for further clinical evaluation. The models and their derivatives demonstrate exceptional accuracy in predicting the pharmacological and toxicological effects of the compound at each organ when all input parameters are well-established. However, variables specific to compounds, such as tissue-to-blood partition coefficients and enzyme kinetic parameters, remain unknown for the new compounds[6]. This poses a significant challenge in obtaining a complete and accurate data set for the model, thus hindering the creation of more precise predictions.

One way to tackle this issue is to fetch data from a model system that closely mirrors the in vivo human body. Theoretically, the greater the resemblance between the two systems, the higher the prediction accuracy.

Currently, animal models are the most used model for the preclinical validation of the tested drug[7]. More than 20 million animal models are serving their duties in the pharmaceutical industry at present[8]. Despite the popularity, animal models have been found to give extremely inconsistent predictions of drug responses in humans[9]. The differences in underlying physiologies between animals and humans explain this. The inadequate matching of genomes may give rise to disparate response mechanisms, consequently yielding misleading physiological outcomes. Hence, doubts have arisen regarding the dependability of animal models as a preclinical platform.

With the major advantage of being homogeneous, patient-derived cell cultures have been emerging as an alternative to animal models for pre-clinical tests. Such a model shares the same underlying molecular mechanism with a real human in vivo environment. Compared to conventional two-dimensional (2D) cell culture where cells are cultured on a planar surface, cell lines cultured in the three-dimensional (3D) way exhibit microenvironments that are way closer to the in vivo conditions[10] by displaying features such as basal polarization, lumen formation, and local cell communication[11]. Facilitated by the incorporation of extracellular matrix (ECM) and biomolecules, in vitro 3D culture techniques enable the co-culturing of

diverse cell types, which spontaneously arrange themselves in a manner that closely resembles the structural and functional characteristics of a specific tissue[12]. By accurately simulating tissue physiology, these artificially engineered 3D tissue cultures, also known as organoids, hold great potential as a reliable tool for predicting therapeutic responses in pre-clinical tests.

Nevertheless, despite their great potential, organoids suffer from several drawbacks. Conventionally, organoids are cultured in a static or semi-static environment without a dispensing system like the blood flow[13]. Diffusion would be the only method for molecule exchange under such an environment. The surface area becomes comparably limited to its volume as organoids grow larger, resulting in an inadequate supply of oxygen and nutrients to the central region of the organoid. In addition, the static environment may also lead to toxic substance accumulation, threatening the biochemical process of the cells. This limitation impairs the viability, functionality, and long-term culture of organoids.

Furthermore, organoids are usually cultured in isolation, whereas in vivo organs interact with one another through physical connections and soluble factors in blood flow. The intricate intercellular communication, coupled with fluid shear force, plays a pivotal role in modulating specific cellular pathways and the production of drug responses. Lack of such cross-organ communication could have a detrimental impact on the replication of overall organ functionality.

To bridge the gap between in vitro and in vivo conditions, attempts have been consistently made to employ microfluidic technology in cell cultures. Microfluidic technology refers to the manipulation and control of small volumes of fluids within microscopic channels, enabling precise handling of liquid flow. Intuitively, this technology is considered a powerful dispensing system that acts as a mimic of blood flow in artificial conditions. By precisely inducing and controlling the microflow that contains nutrients and oxygen as well as metabolic waste, microfluidic devices can enhance the viability and functionality of organoids as all cells are cultured. In addition, the spread of fluid brings the presence of tissue-tissue interaction[14], signaling gradient generation, shear force induction, and perfusion integration in cultured organoids[11]. Organ-on-chip (OoC), a new type of model derived from the incorporation of organoid culture technique and the microfluidic technology, with great advantages in terms of manipulation and physiological relevance, is being proposed as a promising candidate in substituting for animal models during disease modeling and early drug screening.

OoCs are designed from a reductionist viewpoint. It does not aim to create a complete replica of the tissue at its original scale. Instead, this technology strives to reproduce salient aspects of organ structure and function. Engineering OoCs requires knowledge from a broad discipline, including cellular engineering, genetic sequencing, fluid management, biocompatible material fabricating, and live monitoring. This complex nature of its attributes allows a high degree of versatility and can be easily adapted for different organs. By the end of 2021, a significant number of OoC types have been created, including liver[15], lung[16], gut[17], kidney[18], skin[19], bone[20], fat[21], skeletal muscle[22], heart[23], brain-blood barrier (BBB)[24], and tumor[25]. In addition, by exploiting the power of microfluidics, a system of OoCs, where multiple single OoC units are connected via microflows, can be created to simulate the essential functionality of the overall human body[26]. These systems are collectively referred to as body-on-chip (BoC), illustrated in Figure 2. By selectively integrating relevant organ-on-chip (OoC) models, a body-on-a-chip (BoC) platform can be established to express a wide range of system physiology and, therefore, simulate more drug pathways. This approach offers a powerful tool for composing the PK/PD profiles of drug candidates in the pre-clinical stage.

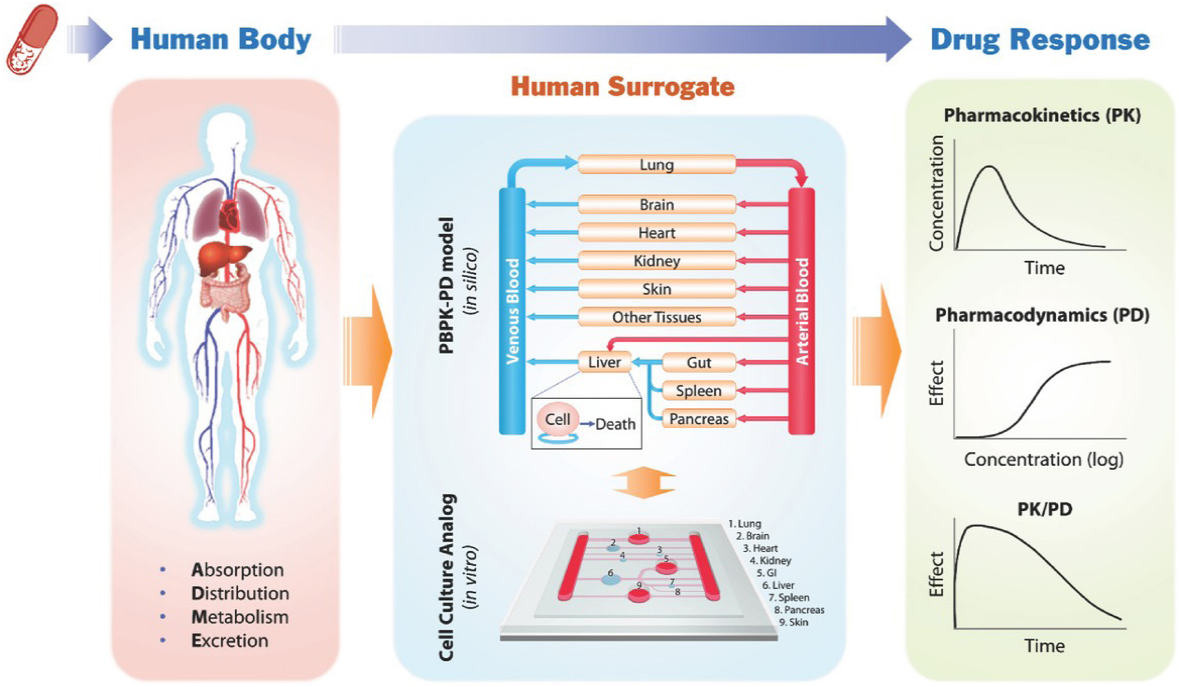

Figure 2. Illustration of cell culture analog, which is an alternative name for OoC/BoC, serving as an in vitro human body representative for PK/PD studies, allowing more accurate predictions of in vivo drug response[27].

As an emerging alternative platform for pre-clinical tests, OoCs/BoCs hold unique advantages as they significantly improve the physiological relevance of in vitro cellular function and morphology, allowing a more accurate representation and prediction of drug-tissue interactions in the real human environment. The OoC/BoC is an interdisciplinary technology that requires the collaboration of multiple subjects. Various aspects must be considered when engineering such a platform, and the design of each chip may differ depending on its purposes. Essentially, the core features of all OoC/BoC devices are universal.

Enigeering OoC/BoC

Cell Source

As a pre-clinical testing platform, cell line obtention is naturally centralized over all other compartments. The three primary sources of cell lines for OoC/BoC manufacture are primary cell lines, immortalized cell lines, and induced pluripotent stem cells (iPSCs). The unique advantages and disadvantages of each cell source are compared in Table 1.

Primary cell lines, as suggested by the name, are the first-hand cells that are directly extracted from target tissue[28]. These cells hold identical genetic expression and physiological properties when placed in the in vitro culture system and, therefore, can perform as a high-quality replica of their original tissue [29]. However, most human organs are located internally. This nature means that cell extraction can be extremely costly and uncomfortable, as surgical processes are considered mandatory when acquiring most types of primary cell lines.

On the other hand, immortalized human cell lines are more economically friendly. By manipulating the cell-cycle checkpoints, the establishment and maintenance of an immortalized human cell line is now a mature process [30]. Although they display some similarity to their in vivo counterparts, the sub-cultured cell lines may branch random genetic variation and evolve new physiologies that may lower their physiological relevance[30].

In recent decades, stem cells have emerged as a new cell source for their versatility. Stem cells can be prepared from various origins, and the obtained cell lines are named after their sources, such as embryonic stem cells (ESCs), mesenchymal stem cells (MSCs), and powerful induced stem cells (iPSCs). Unlike any other stem cells, iPSCs are artificially transformed from specialized somatic cells and rendered the ability to differentiate. This means that it can avoid the ethical or technical issues of obtainment like the other two types yet still hold the same expression profile and pluripotency as they do at the expense of tubes of growth factors[31].

Using this method, easily obtaining adult somatic cells from patients can be reprogrammed and rendered a great ability to differentiate into a substantial number of specialized cell types in the lab. Additionally, multiple

genome editing techniques, such as CRISP-Cas9, viral transfection, and microRNA delivery, can also be utilized to prepare iPSC samples as they allow a precise tailor of cell genomes for obtaining desired functionality [32 33]. The iPSC-derived specialized cells have the same expression profiles as their host cell counterparts and, therefore, can precisely replicate patient-specific cellular physiologies[34]. With this being said, the integration of iPSCs with OoC/BoCs allows the prediction of unique drug responses of that specific patient, leading to a new approach to personalized medicine. iPSCs hold a tremendous capacity to model various functional cells at a precise level and comparably low cost with a bonus of demonstrating patient-specific characteristics. It is now the most popular candidate as the cellular part of OoC/BoC device for pre-clinical drug screening or personalized medicine.

Table 1. Comparison of the performance of each cell type

	Primary Cells	Immortalized Cells	Stem Cells
Source	Tissues	Primary cells	Embryos, umbilical cord, or induced
Lifespan	Limited lifespan	Infinite	Infinite
Genetic Stability	Genetically Stable	May accumulate genetic errors	Can be genetically controlled
Physiological Relevance	Same as in vivo physiology	May deviate from in vivo physiology after generations	High biological relevance
Cost	Costly at the extraction process	Financially friendly	Costly at the culture process

From Cell to Tissue

As mentioned, OoC/BoCs aim to assess drug reactions at a tissue or organ level. Consequently, there's a significant thrust in exploring techniques to fabricate these chips using derived cells.

Living tissues possess a complex 3D architecture where each cell type resides at specific domains, and such organization, referred to as the parenchymal tissue, has been found to be mandatory for any developed tissue models to acquire their functions[35]. Two approaches have been taken to emulate such parenchymal tissue.

The conventional approach in tissue engineering draws inspiration from the intricate process of in vivo tissue morphogenesis, mirroring nature's method by utilizing non-uniform application of growth factors[36]. Cells are meticulously seeded onto a pre-engineered extracellular matrix (ECM) that is typically crafted from porous hydrogels[37]. This ECM serves as a robust scaffold, providing the necessary foundation for cells to adhere and organically construct an initial 3D framework. In addition, the nano-size pores of the material allow a more efficient perfusion and nutrient delivery for internal cell assays. By strategically applying gradients of growth factors across diverse geometric planes, the method orchestrates cell specialization and defines distinct tissue domains[38]. Spontaneous reactions largely drive this process, and this reliance significantly hampers the ability to exercise human control, thereby limiting the fabrication of intricate models that require finer details using this method.

Alternatively, three-dimensional (3D) bioprinting presents a novel avenue. The bioprinting process operates within a highly automated and tightly controlled pipeline, enabling the consistent production of intricate and customizable tissue models with heightened details and organization, as illustrated in Figure 3. Adapted from traditional 3D printing, the 3D bioprinting synthesis tissue model in a layer-by-layer manner[39]. Its workflow commences with the acquisition of an in-depth understanding of the target tissue, including its cellular composition, distribution, and the biomechanical features of the extracellular environment. This is achieved by noninvasive imaging techniques like magnetic resonance imaging (MRI) and computed tomography (CT). The acquired data undergoes computer-aided manufacturing (CAD) for processing, wherein a 3D digital model is meticulously generated based on the captured images[40]. Subsequently, this 3D model is meticulously segmented into numerous 2D cross-sectional slices, each preserving specific anatomical information. The vertical assembly of these slices allows the comprehensive reconstruction of its original 3D structure. Such a digital model is subsequently employed to guide the formulation and patterning of the bioink. Bioink constitutes a tailored blend of porous scaffolding materials, biomolecules, and cells, reflecting the distinctive properties of the targeted tissue[41].

The selection and proportioning of these elements are precisely calibrated to replicate the tissue's unique rheological, mechanical, and biochemical characteristics[42]. Ad-

ditionally, these choices determine the printing resolution, gelling speed, and mechanical and biological attributes of the printing product[43]. Beyond the attributes of the bioink, the actual form of the printed tissue model hinges on the chosen printing approach. The bioprinter lays down the prepared bioink in successive layers, adhering precisely to the specifications outlined in the 3D computer model. Presently, there are four main printing strategies: extrusion-based bioprinting (EBB), inkjet-based bioprinting (IBB), laser-assisted bioprinting (LAB), and stereolithography. Each performs differently in various aspects, summarized in Table 2 below. For its versatility, controllability, and reproducibility, 3D bioprinting is becoming a more preferred approach when engineering functional tissue units for OoC/BoCs.

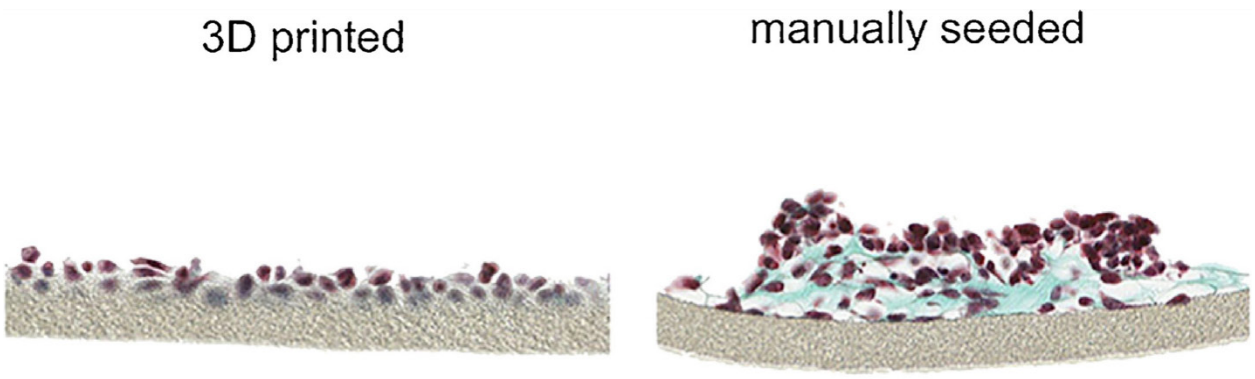

Figure 3. Comparison of tissue structure created from 3D bioprinting (left) and conventional seeding (right).

Brightfield pictures of a two layered air-blood barrier model of 3 days of culture. Where cytoplasm is stained red, collagen fibers of the ECM Matrigel™ green, and cell nuclei dark brown using Masson-Goldner trichrome coloration. The 3D printed model exhibited greater organization and more uniform cell distribution compared to the conventional manually seeded model[44]

Table 2. Comparison of the performance of each 3D bioprinting method[45]

	EBB	IBB	LAB	Stereolithography
Resolution	100 μm	50 μm	10 μm	100 μm
Cell Density	High	Low	Medium	Medium
Cell Viability	89.46 ± 2.51%	80-95%	<85%	>90%
Biomaterials Viscosity	30–6 × 10^7 mPa s	<10 mPa s	1-300 mPa s	No limitation
Printing Speed	Slow	Fast	Medium	Fast
Cost	Low	Low	Medium	High

To establish a singular OoC, the engineered 3D parenchymal tissue is transferred onto the specialized chip containing numerous intricately designed microchambers[46]. These microchambers serve as precise enclosures where the implanted tissue is housed and cultured, immersed in cell culture media that carries essential nutrients. Notably, this media remains in a dynamic state, typically propelled by external forces, ensuring a continuous flow[47]. This flowing fluid enables the constant replenishment of nutrients and removal of metabolic waste, sustaining long-term tissue culture and functionality. For polydimethylsiloxane (PDMS) 's biocompatibility, transparency, and ease of fabrication[46], this material is the primary choice for engineering the chips[37]. PDMS's optical transparency facilitates real-time observation and microscopic imaging of cellular activities and interactions within the chip, allowing rapid data acquisition. Leveraging soft lithography or laser cutting, PDMS's malleability enables the creation of intricate microfluidic channels and structures within the chip, accommodating multiple electrophysical biosensors[48]. These biosensors furnish crucial electrochemical signals, providing insights into cellular metabolites that imaging alone cannot detect[49].

Through the amalgamation and meticulous analysis of

real-time data derived from various sources, the OoC system can construct a comprehensive profile detailing the tissue model's reactions to tested compounds at different stages. This multifaceted approach allows for a thorough examination of the tissue model's intricate responses, capturing its nuanced dynamics under the influence of diverse compounds. This depth of analysis provides the OoC system with a unique advantage, offering detailed insights that are unattainable through conventional pre-clinical models. Such a comprehensive understanding of cellular behaviors and compound responses elevates the OoC's significance in pharmacological studies, enabling a profound examination of complex biological interactions at a level unparalleled by other experimental models.

Connecting Tissue Models

As previously discussed, each administered drug undergoes the ADME process, engaging with different organs in a specific sequence. The compound interacts with the first organ, producing metabolites that subsequently influence downstream organs in a coordinated manner. These metabolites, having originated from the initial organ's reactions, translocate through the vascular system to participate in subsequent interactions with downstream organs[50]. Similarly, the integration of single OoC models through fluidic coupling establishes a mimetic connection, yielding a simplified and miniature representation of the human body in vitro. This amalgamation forms what is referred to as a BoC, proving immensely advantageous for collecting physiological relevant data for PD/PK model calculation at the pre-clinical stage[51].

Scaling System

As an in vitro representative of in vivo organ orchestration, the success of any created BoC system relies on establishing organ models with accurate proportional sizes and vascular volumes, as any imbalance in this aspect could lead to unrealistic concentrations of drug metabolites being transferred between organs. For example, suppose a lung-on-chip, sized at 0.1 μL, were connected to a larger liver-on-chip, measured in mL. In that case, the liver might not respond adequately because the amount of metabolites introduced by the lung is insufficient to trigger biochemical reactions in liver cells. (https://ieeexplore.ieee.org/abstract/document/6428627) Hence, to mimic the evolutionarily optimized in vivo system physiology at various conditions, meticulous calculations are required to be made and adapted to scale the size of each OoC unit in a BoC system.

The most straightforward approach is allometric scaling, in which the biological characteristics, such as organ size and metabolic rate, are scaled by the organism's body mass. It recognizes that as organisms grow larger or smaller, the sizes of their organs or physiological functions do not change in a linear proportional manner[52]. Instead, they change at different rates relative to body size, described as a power law equation where each biological characteristic is proportional to body mass raised to a specific power. The equation is shown below:

$S = a \times (Body\ Mass)^b$

S stands for desired biological characteristics, such as organ mass, oxygen consumption, and metabolic rate. A and *b* are specific constants associated with the studied biological characteristic, the latter of which is also known as the allometric coefficient. The values of the allometric coefficient vary among biological features. For example, roughly metabolic rate, blood circulation time, and vascular networks exhibit scaling of 0.75, 0.25, and 0.75, respectively[52]. Moreover, parameters associated with the same organ may also differ in allometric coefficients. For instance, the allometric coefficients for liver mass, blood flow, blood volume, and oxygen consumption are 0.886, 0.91, 0.86, and 0.69, correspondingly[53]. While this approach of scaling offers a valuable quantitative framework for BoC modeling, it holds several limitations. Mathematically speaking, due to the discrepancies in the M^b term in the equation, it is not possible to construct a BoC system that contains the same proportions of biological characteristics as the in vivo human body. Organs with higher allometric coefficients may scale disproportionately compared to those with lower coefficients, leading to distortions in the relative sizes of organs within the model.

Moreover, biologically speaking, this scaling system ignores a vital aspect highly associated with the ultimate goal of the BoC system, that is, to replicate organ function instead of topology. The equation fails to encapsulate the intricate functional dynamics of organs, such as molecule binding, metabolism, and excretion. Unfortunately, in most cases, such multifaceted organic behaviors hold greater credibility when determining drugs' performance than the sizes of organs do[54].

Hence, a new scaling system, named physiology-based scaling, is developed. Instead of relying solely on body weight, this new scaling system entails a comprehensive consideration of various physiological parameters associated with organ functions[55]. Notably, a complete replication of all functions of an organ is not necessary to determine the size of the organ model. Instead, only the key relative functional aspects are required, depending on the purpose of the engineered chip. For instance, the liver is involved in various metabolic processes, including detoxification, protein synthesis, and nutrient storage[56]. When designing a liver-on-a-chip as a part of a BoC system for drug detoxication studies, focusing on replicating metabolic activities like drug metabolism and toxin breakdown would be adequate in calculating the proper size of the

organ model.

Moreover, additional pharmacokinetic parameters that are not specific to organ functions are also considered. For example, when engineering a BoC system for drug screening, the scaling would consider factors such as target site distribution, target binding and activation, pharmacodynamic interactions, transduction, clearance, and homeostatic feedback mechanisms[57]. By accounting for these variables, physiology-based scaling provides a more accurate estimation of drug activities within the constructed BoC system. With all the variations in design, the sizes of each organ can vary significantly. However, they all share a common characteristic: their sizes are carefully determined from a great amount of meticulous calculations that encompass a wide array of parameters. The results of these calculations will set the stage for the eventual coupling of organs to form a BoC system.

Direct Coupling

To couple each organ model, the most straightforward approach is direct coupling, in which all the organs are cultured in the same media, and the metabolites from one organ chamber are directly delivered to the next organ chamber by one continuous fluid flow[58]. The microflow allows the transportation of metabolites in a rational order, replicating the vascular system[52]. An adequate amount of microflow that contains nutrients and drug samples is constantly perfused into the system by external pumps and eventually leaves the system with deposits. The patterning and dispensing of the microflow is achieved by the integration of microchannels on the chip.

As previously mentioned, PDMS is particularly favored in the engineering of OoC/BoC devices compared to the other available polymers such as polycarbonate (PC)[59], polyimide (PI)[60], poly(methyl methacrylate) (PMMA)[61], and the cyclic olefin polymers (COC) family[62]. This is because PDMS offers a distinct advantage due to its ease of fabrication. Depending on the methodologies, microchannels can be fabricated using two different approaches on the PDMS chip.

The first approach is photolithography, shown in Figure 3. Due to the presence of an inorganic siloxane backbone and organic methyl groups attached to silicon, the PDMS material is in the liquid state at room temperature while holding a low glass transition temperature[63]. Such properties make the use of a stamp, known as the master mold in this process, particularly effective when crafting microchannels on the material[63]. The master mold is a template that dictates the microchannel pattern and is created through photolithography using a photosensitive material. By selectively exposing photoresists that are cast on a silicon wafer to light through a photomask that contains the desired pattern, the desired geometry of protrusions will form on the surface of the master mold[63]. Subsequently, a liquid PDMS pre-polymer is poured onto the master mold and applied to cure agents to initiate the crosslinking process. After curing, the PDMS replica is peeled off the master mold, resulting in a flexible and transparent elastomeric structure with the pre-designed microchannels inside. The master mold can then be recycled and reused to produce more chips with patterned microchannels.

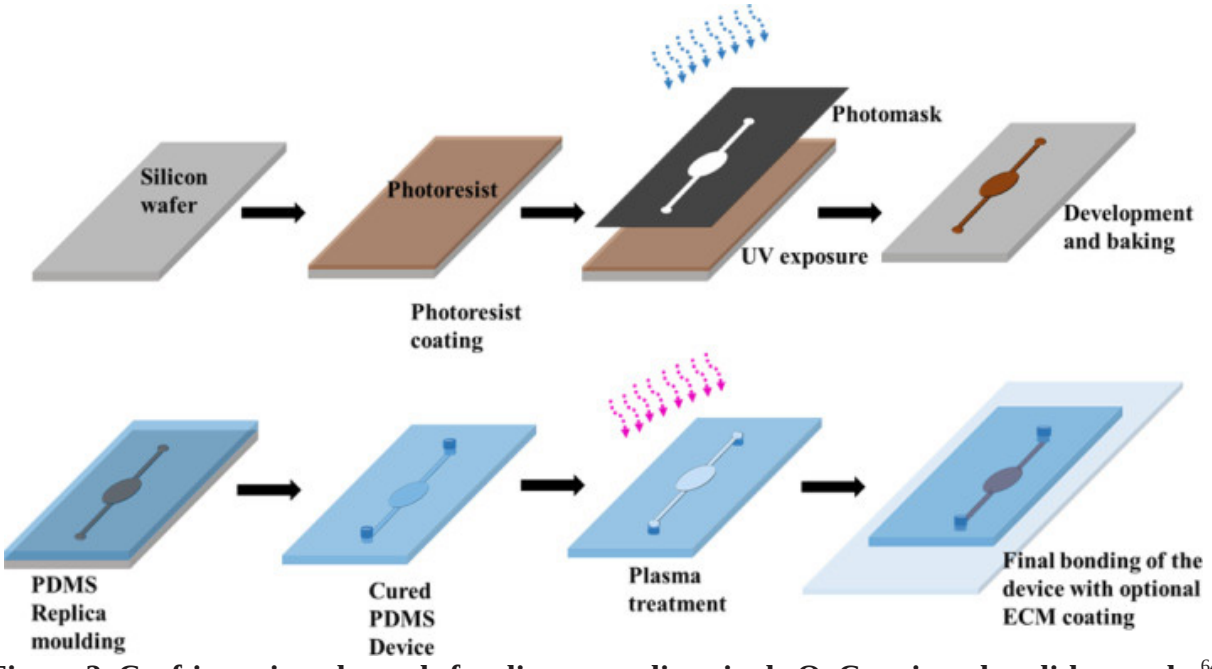

Figure 3. Crafting microchannels for direct coupling single OoCs using photolithography[64]

An alternative way to fabricate microchannels is laser cutting. This method involves using a laser beam to directly create microchannel arrays in a cured PDMS layer[65]. After curing, a laser, controlled by computer software, is directed onto the PDMS surface to precisely ablate the material, forming the desired microchannel patterns. The intensity and focus of the laser beam can be adjusted to control the depth and width of the channels[66]. Compared to soft lithography, which requires access to cleanroom facilities and specialized equipment, laser cutting offers rapid prototyping capabilities, flexibility in design, as well as financial advantages. However, due to the width of the laser beam, the resolution of the created microchannel using laser cutting may not be as precise as photolithography techniques used in soft lithography, which can achieve extremely fine patterns.

After fabricating the PDMS layer with open channels, it is then bonded to a substrate for sealing. This is typically achieved through oxygen plasma bonding. In this method, the surfaces of the PDMS pieces to be bonded are exposed to oxygen plasma, which generates reactive oxygen species on the surface. These oxygen species react with the PDMS surface, creating silanol (Si-OH) groups. When two treated PDMS surfaces are brought into contact, the silanol groups form covalent bonds between them through a condensation reaction, resulting in a strong and permanent bond[67].

Then, depending on the purpose, the material is treated with various chemical surface modifications; details are described in this review by Shakeri et al[68]. Moreover, biological modifications can also be applied to better mimic blood vessel physiology. For example, Kihoon Jang et al reported their work to culture endothelial cells (ECs) within the microchannel surface. In exposure to UV light, they successfully developed a technique to grow endothelial cells (ECs) and MC-3T3 E1 cells on the surface of microchannels, thus improving the physiological relevance of the microchannels[69].

Pumps of various sizes normally drive the fluid through channels at a rate that allows sufficient material exchange between cultured cells and the fluid. Pumps can be roughly divided into two categories: mechanical displacement pumps and energy transfer pumps[70]. Mechanical displacement pumps utilize the repetitive mechanical movement of diaphragms or flaps to drive fluid movement. This motion is typically generated through various actuation mechanisms, including mechanical, piezoelectric, thermal, and pneumatic methods[717273]. On contrary, energy transfer pumps induce fluid flow by directly transferring energy to the sample fluid, exampling by the electrohydrodynamic[74], magnetohydrodynamic[75], electrochemical[76], and ultrasonic pumps[77]. These pumps are generally regarded as offering more consistent and precise control over fluid flow compared to mechanical displacement pumps. However, they are often accompanied by the drawback of being more expensive. Aside from these conventional pumping approaches, recent work proposed a new way of generating fluid flow without pumps. Due to their special geometry, the fluid can spontaneously generate flow with the assistance of capillary suction[78] or gravity[79].

By altering fluid velocity and channel geometry, a stable BOC system can be established from directly coupling OOC units, allowing the replication of physiological processes that involve multiple organs working in concert, providing a more holistic understanding of drug ADME, efficacy, and toxicity. However, direct coupling poses challenges in scalability primarily due to its inherent limitations in maintaining independent microenvironments for each organ model and regulating inter-organ communication.

With direct coupling, all organ models are physically interconnected, resulting in shared culture media and experimental conditions across the entire system. This interconnectedness restricts the ability to independently adjust experimental parameters, such as flow rates, nutrient concentrations, and drug doses, for individual organ models[80]. Failure to account for these variations can result in inaccurate simulations of interorgan interactions and responses to drugs or toxins, as well as shortening the functioning date of the BoC system. Moreover, the flexibility of this method is doubted when creating a complex BoC system with multiple organ models. As the number of interconnected organ models increases, the complexity of the system also grows significantly. Directly coupling multiple organs requires precise engineering to ensure proper fluidic connections, control over environmental conditions, and compatibility between different organ models. This complexity can pose challenges in terms of experimental setup, operation, and data interpretation.

Additionally, the rigidity of direct coupling may hinder the ability to modify or reconfigure the BoC system according to evolving research needs or experimental conditions. Researchers may encounter difficulties in integrating new organ models or adjusting experimental parameters.

Meanwhile, any malfunction of any unit will affect the whole system and eventually produce the same outcome. The physical connection makes it impossible to fix the malfunctioning unit individually in isolation; the whole BoC system will have to be discarded. With all being said, the cost and resources required to develop and maintain a complex BoC system through direct coupling can be substantial. To address these limitations, functional coupling presents a viable alternative, offering more flexibility and versatility in controlling inter-organ interactions.

Functional Coupling

As an alternative to direct coupling, functional coupling addresses the prior's limitations effectively as it focuses on emulating the biochemical and signaling cascades that regulate organ-to-organ communication. In this approach, each organ model is cultured in separate chambers, allowing the creation of organ-specific physiological microenvironments. The compound under investigation is initially applied only to the chamber containing the first organ involved in the ADME process. After their interaction, key metabolic compounds crucial for the downstream ADME process are extracted from the chamber. These extracted compounds are then introduced into a media, which is dynamically adjusted based on the metabolic activity of the subsequent destination organ model. The selection of the destination organ model is determined by the physiological sequence within the human body. Subsequently, the media containing the modified compounds is transferred to the next organ model. This process of adjustments and transfers is repeated until the ADME process is complete[58]. The precise arrangement of the organ modules and the sequence of transfers may vary according to the specific objectives of the BoC system. Still, the dynamic nature of the connections remains consistent across applications.

Functional coupling presents a significant advantage over direct coupling due to its flexibility and versatility, as demonstrated in Figure 4 below. Unlike direct coupling, which requires all organ models to operate simultaneously, functional coupling allows for the independent operation of each module. This enables researchers to assess the performance of individual organ models separately, enhancing the efficiency of experiments and enabling more targeted analysis. Additionally, the ability to adjust solute types and concentrations at each module eliminates the need for a universal culture solution, simplifying experimental setup and interpretation. Furthermore, the modular nature of functional coupling allows for dynamic combinations of organ models, providing researchers with greater flexibility in designing experiments and addressing specific research questions. For instance, in a study conducted by L. Vernetti et al. in 2016, liver and muscle models were initially functionally coupled to assess the toxicity of terfenadine. Subsequently, gut and BBB models were seamlessly integrated into the system to investigate the chemical's absorption and penetration without redesigning the whole BoC system or reengineering the other organ modules, demonstrating the adaptability and versatility of functional coupling[58].

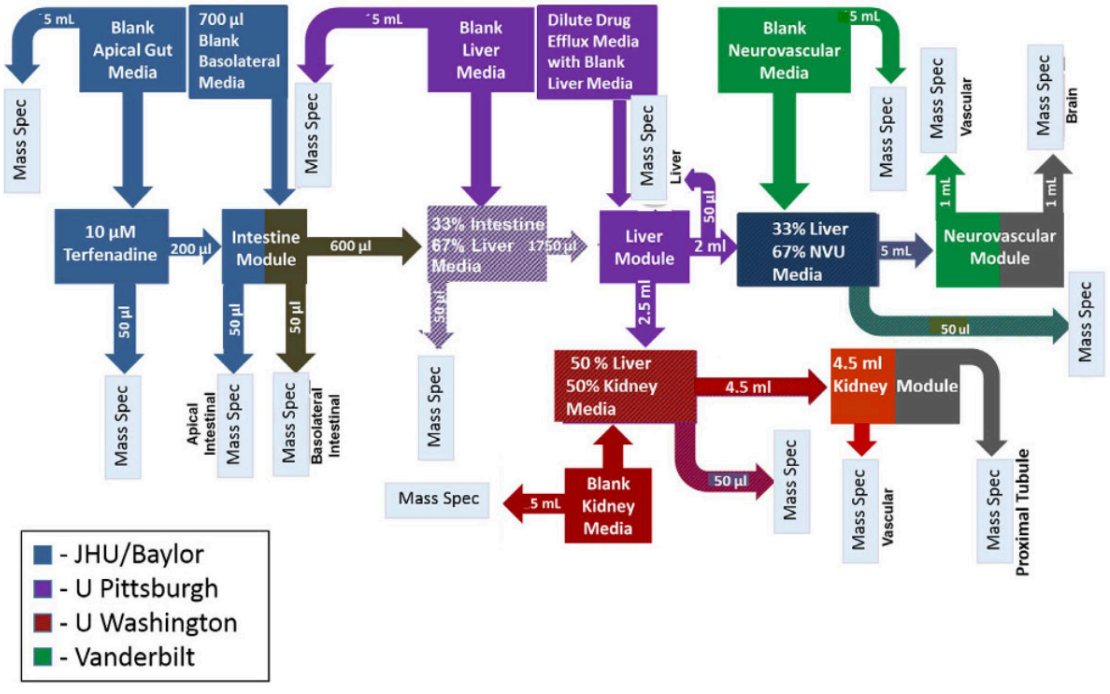

Figure 4. Workflow of a functional coupled BoC system

The BoC system contains gut, liver, kidney, skeletal muscle, and neurovascular module and is used to test the behavior of terfenadine, trimethylamine (TMA), and vitamin D3. Noticeably, specific paths within this workflow are chosen for evaluating each compound; the specific purpose of the test determines the selection. In addition,

this BoC system was created by the collaboration of four universities, and each contribution is represented by the four different colors shown in the figure. This collaborative endeavor demonstrates the remarkable flexibility and versatility of functional coupling.[58]

Application of OoCs/BoCs

Toxicity and Efficacy

Evaluation of toxicity and efficacy is of paramount importance in the preclinical stage of drug development due to the frequent occurrence of unexpected toxicity or unsatisfying efficacy of the candidate detected during phase III clinical trials or even post-marketing[81]. It is vital to gain toxicological and performance evaluations before investing more money to conduct further studies. Since 2010, nearly all human organ systems have been translated into OoCs[82]. Such a well-developed platform has profoundly facilitated the study of the characteristics of any organ system, including their physiological processes and their responses to xenobiotics. Naturally, they become the perfect candidate for assessing organ-specific toxicity and efficacy in the context of drug development. The toxicity or efficacy of various substances, ranging from traditional chemical drugs to newer modalities like immunotherapy, radiation therapy, nanomedicine, and interactions involving the microbiome and host, has been assessed on single OoCs[83].

The OoC platforms provide a physiologically relevant environment by mimicking the microarchitecture and cellular interactions of human organs. This enables more accurate predictions of drug responses and toxicity levels, facilitating better-informed decisions in drug development. Despite the advantages, single OoCs ignore the fact that a drug is normally processed by other organs, typically the liver, before being dispensed to their targets. By the time drugs reach their targets, the liver may have already altered their biochemical properties. Hence, it is crucial to account for these upstream modifications when investigating xenobiotic toxicity and efficacy at the target organ. As a result, the liver, as the center of exogenous molecule metabolism, is often associated with other OoCs, creating a BoC system to examine the potential toxicity and efficacy of the drug.

PD/PK

As mentioned in previous sections, PD/PK studies are pivotal in the preclinical stage as they provide crucial insights into the interactions between drugs and the body system. They provide instrumental information on the pharmacological responses that may occur, thus assisting in minimizing and the side effects of drugs by adjusting the dosage[84]. In addition, by determining the optimal dosage, route of administration, and dosing frequency, PD/PK investigations help inform the design of future clinical trials, ensuring that experimental drugs are evaluated under conditions conducive to favorable outcomes. Moreover, the PD/PK data gleaned from preclinical studies guide the selection of appropriate patient populations and endpoints for clinical trials, thereby enhancing the likelihood of successful translation from preclinical research to clinical practice[85]. In contrast to toxicity and efficacy studies that focus on single-organ responses to the drug, PD/PK studies aim to investigate the systematic responses of the whole body that involve multiple organs. This nature precisely aligns with the strength of the BoC technology, which is the recreation of the dynamic interactions among organs, and thereby establishing a faithful depiction of systemic physiological reactions. Moreover, the modular structure of BoCs permits tailored experimental setups, allowing a flexible in examining drugs that hold differences in targets and ways of administration.

Personalized adaptions

Over centuries of medical practice, it has become increasingly evident that individuals exhibit variations in gene expression profiles, leading to diverse responses to the same drug or therapy. Naturally, treatment accuracy and effectiveness can be significantly improved by stratificating patients into groups based on their genomic signatures for group-specific treatment[86]. The concept of personalized medicine has emerged following this logic, aiming to tailor prediction, prevention, and treatment strategies to individual patients' illnesses[87]. Leveraging OoC/BoC technology presents a promising approach to identifying these variations and enhancing the efficacy of prescribed drugs. By utilizing patient-specific cellular compartments that match the genomic signatures of specific population groups or individuals, such as stem cells, personalized OoCs/BoCs can be engineered. Such devices allow healthcare providers to customize prescriptions to enhance treatment effectiveness by enabling personalized drug response prediction, including its toxicity and efficacy, PD/PK, and combination therapy.

Firstly, it enables patient-specific drug toxicity and efficacy predictions. By recreating the unique microenvironment of an individual's organs, including genetic variations and disease phenotypes, personalized OoC models allow for precise examination of toxicity and efficacy. Clinicians can use these models to anticipate how an individual will respond to various medications and identify potential adverse effects or inefficacies before treatment initiation. Additionally, personalized OoC platforms facilitate precision medicine approaches by enabling the

simultaneous testing of multiple drugs or drug candidates, providing valuable insights into the most effective treatment options based on an individual's biological profile.

Personalized PD/PK studies can also be realized using personalized OoCs to optimize treatment regimens. These platforms allow for the simulation of diverse drug dosages and treatment schedules, empowering clinicians to tailor treatment regimens to maximize efficacy while minimizing side effects. Real-time monitoring of drug effects within personalized OoC models further permits dynamic adjustments of treatment regimens based on individual patient responses, ensuring that treatments are continually optimized over time to achieve the best outcomes for each patient.

Finally, these platforms also play a critical role in evaluating combination therapies on individual patients, particularly for treating complex diseases such as human immunodeficiency virus (HIV) infections, hepatitis C virus (HCV) infections, and cancer[88]. These conditions are characterized by multifactorial pathologies, necessitating the collaboration of multiple drugs targeting different pathways to achieve maximum efficacy[89]. Moreover, these diseases can exhibit considerable patient-specific variability, resulting in challenges in uniform treatment outcomes[90]. By co-culturing multiple organ models, researchers can assess the interactions between different drug combinations, evaluating synergistic effects and compatibility. This enables the prediction of personalized combination therapies that are most effective for individual patients while also allowing for the proactive identification of potential adverse drug interactions based on patient-specific factors such as genetic variations and metabolic profiles.

Opportunities and Obstructions

The OoC/BoC technology, as described above, has shown its great potential in the drug development pipeline and personalized medicine. It holds the capability to establish one of the most physiologically relevant in vitro systems that far exceed the traditional models. Such mimicry allows this platform to be particularly suitable for conducting studies about humans, especially those which are difficult to conduct in humans, for example, lethal toxin levels. Simply by establishing a BoC system, combined with mathematical translation, the result can be easily obtained without any ethical concerns being raised. Moreover, due to its versatility, nearly all combinations of organs, can be created as OoCs with any available desire genetic constituent. This is an extremely powerful attribute as it allows a very broad context of use across the entire drug development pipeline and its administration. For example, in pre-clinical stages, drug safety and drug efficacy can be more accurately predicted using OoCs/BoCs compared to animal models since the prior is made of the same genetic material as humans. While OoC/BoC systems typically entail higher costs compared to traditional models[82], their enhanced precision offers greater efficiency in selecting drug candidates for later stages of development. This precision helps avoid the expense of testing potentially unsuccessful compounds that may go undetected in animal models from entering the more costly clinical trials, thereby reducing the overall cost of drug development. By estimation, 25% of research and development expenses can be saved by integrating the OoC/BoC platform in the drug development pipeline[91]. An improved efficiency leads to increased productivity; a recent analysis showed that solely by replacing animal models with liver OoCs, an annual extra US$3 billion can be generated for the pharmaceutical industry[92]. Noticeably, the OoC/BoC technology is still at its young ages, the cost to manufacture each device is expected to gradually decrease with the maturation of relative industries, such as material fabricating and cellular part harvesting. Currently, the most promising applications of OoCs/BoCs lies in the pre-clinical stages. However, the potential of the technology is driving it gradually towards the later stages of the drug development pipeline. With proper advancements in multiple disciplines, the ultimate goal of the OoC/BoC should be to provide an alternative to certain parts or most parts of the clinical trials.

In addition to its benefits in integrating into the drug development pipeline, it is reasonable to expect more from this technology for its potential application in personalized medicine. By using patient-specific cells, each OoC/BoC device can be tailored into an in vitro representative of that specific person. Consequently, studies and experiments that are difficult to conduct within the human body can be effectively carried out on these devices. Given the variability in individual responses to therapy, the most significant application lies in their potential to function as personalized laboratories, providing dosage and drug selection guidance to healthcare providers such as doctors and therapists. This can be integrated into the hospital system as a part of clinical laboratory assessment, where the chip is created in accordance with each patient's will at an extra charge. By analyzing data from the personalized chip, healthcare providers can identify optimal drug combinations and dosages, ultimately enhancing treatment efficacy. This approach could be particularly impactful in the treatment of complex and severe diseases like cancer, where therapies can be costly and may not yield the expected results. Utilizing such platforms may help eliminate ineffective treatment approaches and save patients from unnecessary pain and therapeutic expenses.

Despite the big future, the biggest challenge impeding this

technology from occupying the majority of the market is gaining acceptance and validation of the results obtained from this technology from interested parties, such as pharmaceutical companies, regularity agents, and academic research groups. This process can be long and complex. Straight evidence of comparable or enhanced effectiveness compared to animal models will be necessary. To obtain such evidence, a universally accepted and applied criteria that assess the performance of any OoC/BoC system, allowing the demonstration that this technology can generate more physiological relevant data consistently and statistically better than conventional models[93]. Currently, most OoC/BoC systems are designed by individual labs, which display great variations in design and manufacturing. However, to make reliable demonstrations, extensive assessment on a large scale involving hundreds of devices of identical design operated according to uniform protocols is required. This brings new challenges to designing a universal engineering protocol that standardizes the production of the device, involving the large-scale production of desired cell types, culture media, materials, and operation methods. Luckily, international organizations such as international organizations, like the microphysiological systems affiliate of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ) Consortium and the US Food and Drug Administration (FDA), have established a list of qualification terms to examine the performance of drug screening platforms, including the OoC/BoC technology[94]. Basic benchmarks have been created in the guidance of designing and manufacturing such devices, and the width of such qualifications continues to expand. Currently, the OoC/BoC platform is at a point where a brighter future is within reach. In the coming decades, this technology may gradually dominate the market of drug development and personalized medicine as a more effective substitute for conventional animal models.

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