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Review

From Organ-on-a-Chip to Human-on-a-Chip: A Review of Research Progress and Latest Applications

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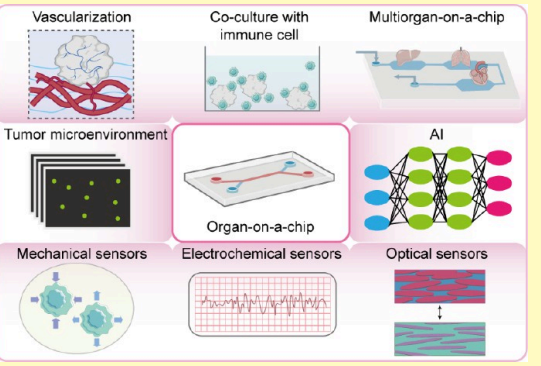
ABSTRACT: Organ-on-a-Chip (OOC) technology, which emulates the physiological environment and functionality of human organs on a microfluidic chip, is undergoing significant technological advancements. Despite its rapid evolution, this technology is also facing notable challenges, such as the lack of vascularization, the development of multiorgan-on-a-chip systems, and the replication of the human body on a single chip. The progress of microfluidic technology has played a crucial role in steering OOC toward mimicking the human microenvironment, including vascularization, microenvironment replication, and the development of multiorgan microphysiological systems. Additionally, advancements in detection, analysis, and organoid imaging technologies have enhanced the functionality and efficiency of Organs-on-Chips (OOCs). In particular, the integration of artificial intelligence has revolutionized organoid imaging, significantly enhancing high-throughput drug screening. Consequently, this review covers the research progress of OOC toward Human-on-a-chip, the integration of sensors in OOCs, and the latest applications of organoid imaging technologies in the biomedical field.



KEYWORDS: *organ-on-a-chip, human-on-a-chip, vascularized OOCs, multiorgan-on-a-chip, sensors integrated in OOCs, organoid imaging, artificial intelligence, drug screening*

The process of developing new drugs is both lengthy and costly. Traditional biomedical research and development has historically relied on analyzing animals at molecular and cellular level. However, due to the uncertainties surrounding the toxicity and effectiveness of new drugs, extensive testing within cell lines and animal models is required before human trials can commence. Unfortunately, the physiological response of cell lines and animal models differs significantly from that of humans.[1] As a result, there is a pressing need for a disease model with human relevance in order to reduce the cost and duration of new drug development. In a significant development in December 2022, the U.S. Congress approved the FDA Modernization Act 2.0, which eliminated the mandatory requirement for animal tests of new drugs and encouraged the gradual adoption of alternative models when possible. This milestone event highlighted the FDA's strong endorsement of the credibility of research on organoids.[2] The innovative development of organoids has made it feasible to replace animal experiments.[3]

An organoid is a three-dimensional (3D) tissue formed by stem, progenitor, or differentiated cells that self-organize through cell sorting and space-constrained lineage differentiation.[4] Organoids offer several advantages, such as short in vitro operation cycle, high similarity to human tissues and structures, molecular characteristics, and drug reactions. This makes them a promising platform for basic research, drug screening, and precision medicine, potentially revolutionizing drug development and clinical treatment options.[5,6] Despite their ability to closely mimic human organs, organoids have limitations in achieving prominent bionic performance, controllability, and reproducibility. This has led to the emergence of Organ-on-a-Chip (OOC) technology. OOC is designed to contain multiple cell types specific to an organ, closely resembling the histological and genotypic characteristics of human organs and partially replicating the specific physiological functions of the organ.[7] OOC can be used to construct tissue and organ microenvironments in vitro, incorporating various living cells, functional tissue interfaces, biofluids, mechanical stimulation, and other complex factors. This sophisticated microenvironment mirrors the main structural and functional characteristics of human tissues and organs and can be applied to develop disease models, new drugs, immune response therapy, microbial infections, and more. It also enhances predictability in clinical therapeutic solutions and

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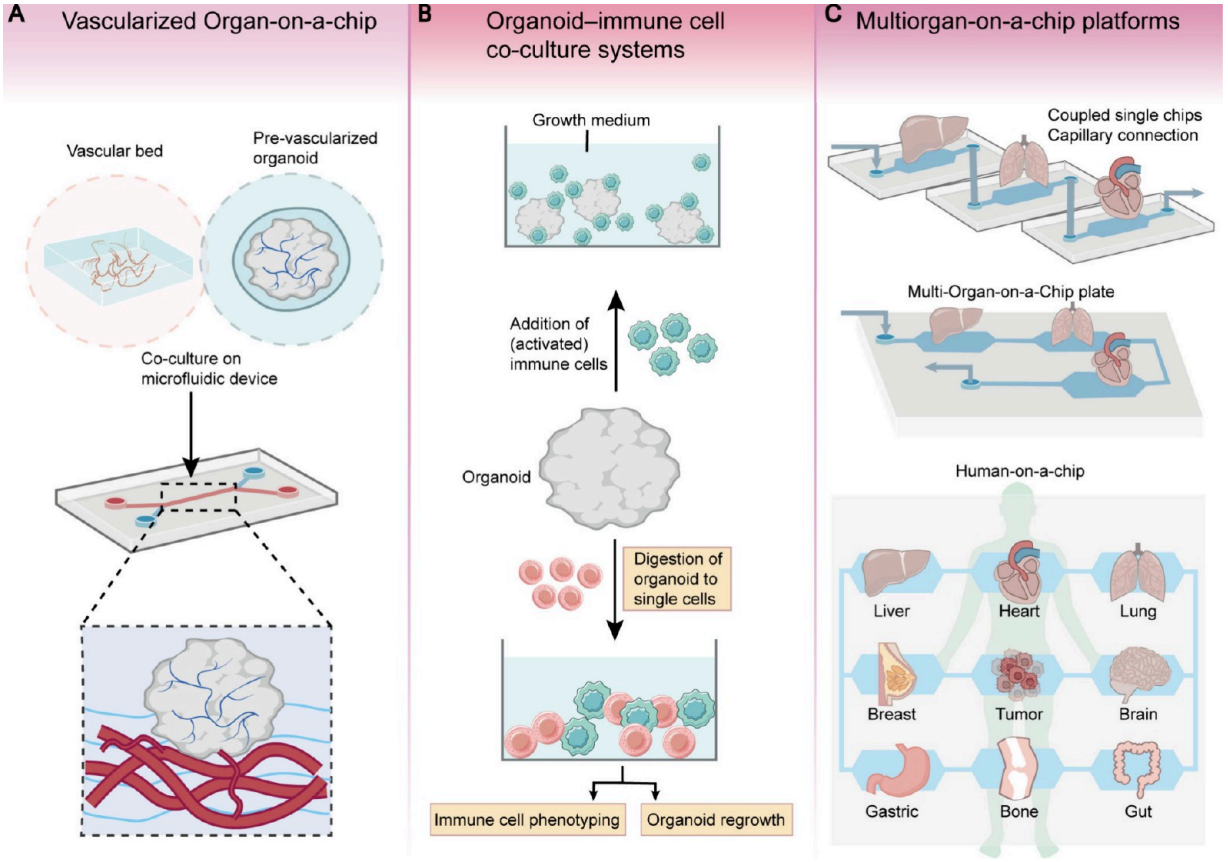

Figure 1. Functionalization of OOCs. (A) Mechanisms for establishing anastomoses between in vitro vascular beds and prevascularized organoids in coculture on microfluidic chips. (B) Two organoid-immune cell coculture systems used in basic research to study immune cell and epithelium interactions. Addition of (activated) immune cells to intact organoids in growth medium (suspension culture) is used to assess the interaction between immune cells and epithelial cells, or the organoids are digested into single cells and then regrown in the presence of immune cells. (C) The primary approaches to the development of multi-OOC platforms. Coupling individual OOC via capillary connections or microfluidic motherboards, each modeling a different organ, or integrating different organoid models on a single plate, which most closely approximates the human-on-a-chip concept.

significantly improves the efficiency of research experiments.[8−10]

The development of Organs-on-Chips (OOCs) has encountered several challenges similar to those faced by organoids. While OOCs are designed to mimic human physiological processes, replicating the full complexity of human physiology, including vascularization, simulating the human microenvironment, real-time detection, and organoid imaging, remains a significant challenge in implementing the concept of Human-on-a-Chip.[11] However, recent efforts and progress in these areas have been more sustainable. This paper reviews the latest research progress in vascularized OOCs, OOCs simulating tumor microenvironments, Multiorgan-on-a-Chip (multi-OOC) systems, and integrated biosensor organoid systems. It also introduces the applications of these microsystems in drug testing, screening, and discovery, as well as personalized medicine. Furthermore, the paper discusses the existing challenges and prospects of artificial intelligence (AI) technology in the field of organoid imaging to promote its application in various biomedical research areas.

■ ORGANS-ON-CHIPS

The OOC is a flexible microfluidic device designed to replicate the microstructures and microenvironments of human organs. It achieves this by utilizing micronano precision machining and microfluidic analysis technologies to mimic the complex microphysiological functions of organs in vitro.[12] The OOC incorporates multisensing, multiorgan, and an embedded system to dynamically monitor and control mechanical stimulation, physiological signals, spatiotemporal chemical gradients, and tissue interface interactions with other organs, thus creating a comprehensive bionic human system.[13−15] When exposed to mechanical stress from fluid flow and circulation, the cells experience environments that closely resemble those within the human body, such as intestinal peristalsis, pulmonary respiration, and blood flow in blood vessels. The OOC also offers advantages in reproducing more complex structural features, including multicellular complexity, intercellular interactions, specific tissue extracellular matrix (ECM), and mechanical forces.[16] Compared to traditional cell culture methods, the OOC shows greater promise in replicating human physiology in terms of structure, morphology, gene expression, and organ function in vitro.[17]

■ VASCULARIZED ORGAN-ON-A-CHIP

Currently, most organoids do not have vascularized structures, which hinders their growth as the organoid volume increases. This is due to a lack of oxygen and a buildup of metabolic waste, leading to an increased risk of tissue necrosis.[18] The circulatory system plays a crucial role in oxygen exchange and mass transfer, highlighting the need to incorporate it into OOC technology to recreate the microenvironment at the tissue and organ level

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while preserving essential physiological functions.[19] The advancement of OOC has led to the integration of vascularization into in vitro organoids on chips (Figure 1A). Vascularized OOC offers a more physiologically relevant in vitro system,[20] particularly in the vascularization of liver-on-a-chip, lung-on-a-chip, and heart-on-a-chip, which is vital for drug screening and personalized medicine.[21]

Vascularized Liver-on-a-Chip. The liver is a complex and highly vascularized organ consisting of 5 major cell types: hepatocytes, Kupffer cells, hepatic stellate cells, cholangiocytes, endothelial cells, and various circulatory immune cells.[22] These cells are essential for the liver's functions of nutrient metabolism, drug detoxification, and immune response.[23] While replicating hepatic zonation with human hepatocytes in traditional static 2D cell cultures is challenging, the development of liver-on-a-chip technology has greatly facilitated the process. Liver-on-a-chip can effectively mimic both the function and structure of the human liver, which is crucial for research on liver disease and pharmaceutical development.[24] Moreover, accurately reconstructing the specific structural features and surrounding microenvironment, including the vasculature with a continuous oxygen supply, is crucial for in vitro liver modeling.[25,26] Although various liver chips have been developed using emerging OOC technology, replicating hepatic lobules with a self-assembled network of perfusion hepatic sinusoids remains a significant challenge. A bionic liver lobule chip featuring a perfusion-capable hepatic sinus network created using a microfluidic-guided angiogenesis method has shown promising results. Compared with other liver chip technologies, this liver lobule design has demonstrated the ability to produce more biologically relevant liver microstructures, higher metabolic capacity, and more durable liver cell function, offering new insights for the design of broader bionic vascularized liver chips.[27]

Researchers have successfully developed a chip-based model of vascularized hepatocellular carcinoma that can replicate albumin secretion and in vivo-like vascular permeability for measuring transport.[28] To mimic the liver microenvironment, they cocultured induced hepatic (iHep) cells with vascularized endothelial cells, creating a 3D model using liver-specific ECM hydrogel and microfluidic technology for media flow. This advanced 3D iHep culture system shows higher sensitivity and less variation in hepatotoxic reactivity, making it highly suitable for drug toxicity research.[29] While there have been recent advances in scalable 3D liver scaffolds for drug metabolism and toxicology studies, standardized test platforms aimed at replacing animal models are still in the early stages. The main challenge lies in the lack of accurate methods for specific predictions and the repeatability of data-driven results.[30] The newly developed liver model not only demonstrates high predictability but also scalability, making it easily adaptable to high-throughput drug screening and implantation research. It provides a promising alternative to animal models.[31] Furthermore, a microfluidic platform known as structurally vascularized hepatic ensembles for analyzing regeneration (SHEAR) has been developed. SHEAR can simulate various aspects of human liver regeneration by controlling hemodynamic changes, simulating liver injury and changes that occur during regeneration, and facilitating biochemical inputs such as cytokines and paracrine interactions with endothelial cells.[32]

In recent years, there has been a shift in the construction of in vitro vascularized liver tissue. This tissue has a wide range of biomedical applications, including liver regeneration, disease modeling, and drug screening. Advancing this field requires diverse cell sources and appropriate biological materials for damage repair and drug toxicity research.[33] Future developments may focus on creating devices that incorporate all cell types found in the liver. Additionally, real-time monitoring, as opposed to end point analysis, will provide a more comprehensive understanding of the dynamic changes in these devices.[34]

Vascularized Lung-on-a-Chip. Given the current coronavirus pandemic, there is an urgent need for a model that can effectively elucidate viral pathogenesis in the lungs. This underscores the potential utility of lung-on-a-chip models. The development of the vascularized lung-on-a-chip presents significant potential in the fields of tumor microenvironment engineering, drug screening, and combating virus epidemics.[35] This technology facilitates the transport of anticancer drugs and immune cells within the tumor microenvironment, while also enabling rapid screening of existing drugs for the prevention and treatment of viral outbreaks such as COVID-19.[36,37] The inclusion of vascular regions maintaining blood flow and the replication of the air−liquid interface, which are crucial physiological features lacking in organoids, makes this innovation particularly promising.[38] The human alveolar-capillary barrier, essential for gas exchange and protection against external hazards, is faithfully replicated through the synergistic interaction of human epithelial−endothelial cells within a 3D ECM. This provides a reliable research platform for accelerating the effective treatment of COVID-19.[36]

Notably, the pioneering work of Huh and colleagues in 2010 established an OOC model of the human alveolar-capillary interface, demonstrating the potential of this technology to mimic lung physiology in vitro.[39] A decade later, Min Zhang et al. demonstrated the utility of microengineered alveolar chips in modeling lung injury and the immune response induced by natural SARS-CoV-2. These bionic systems successfully recreate key characteristics of the human alveolar-capillary barrier through the coculture of alveolar epithelial cells and microvascular endothelial cells in a microfluidic state. Studies have indicated that treatment with the antiviral compound remdesivir can effectively suppress viral replication and mitigate the disruption of alveolar barrier integrity caused by viral infection.[40] Additionally, a chip model infected with the influenza A virus has shown promise in supporting the differentiation of lung bronchial airway basal stem cells into cell types with airway specificity, thereby enhancing the expression levels of TMPRSS2 and ACE2.[41] However, the absence of clinical trials necessitates caution, as the potential for confounding and selection bias cannot be ruled out. Therefore, it is imperative to verify these findings in prospective double-blind clinical trials to fully evaluate the efficacy of these interventions.[42]

Vascularized Heart-on-a-Chip. The heart is a dynamic multicellular organ with a compact structure that requires a substantial amount of energy to transport blood carrying nutrients, oxygen, and metabolic wastes to other parts of the body. Its role in generating aerobic pumping movement is vital. The myocardium plays a direct role in the contraction and relaxation cycle, which has sparked interest in researching the construction of myocardium in cardiac organoids in vitro.[21] Myofibroblasts exhibit characteristics of both fibroblasts and smooth muscle cells. They produce ECM and have the ability to initiate contractions. Identifying the molecular targets of myofibroblasts and determining the feasibility of molecular imaging of these cells may contribute to early detection and

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treatment in patients at risk for heart failure after myocardial infarction.[43] However, the diffusion process for providing oxygen and nutrients to the cardiac microtissue is limited. To improve the clinical feasibility of the tissue model, thicker tissue needs to be constructed, which would involve producing a capillary network within the tissue to deliver nutrients to the cells.[44]

The current utilization of microfluidic technology has enabled the development and replication of the primary cardiovascular environment. These novel microfluidic systems have broad applications in natural organ simulation, disease modeling, drug screening, disease diagnosis, and treatment, facilitating the study of potential mechanisms of cardiovascular diseases.[45] The use of human-induced pluripotent stem cell cardiomyocytes (HiPSC-CMs) in tissue engineering is poised to revolutionize drug discovery.[46] Additionally, a cardiac fibrosis model on a chip has exhibited transcriptome features consistent with human cardiac fibrosis/heart failure, enabling the testing of antifibrosis drugs and facilitating real-time assessment of myocardial cell function.[47] HiPSC-CMs offer an opportunity to gain deeper insights into the pathophysiological mechanism of ischemic heart disease (IHD) and to serve as a platform for drug screening. The antiarrhythmic effect of levosimendan was clearly observed in an in vitro model of IHD using HiPSC-CMs under hypoxic conditions, pointing to potential new clinical applications for the drug.[48]

The potential benefits of microfluidic platforms for improved physiological correlation and increased throughput over traditional cell culture techniques are significant. However, there is still a need for microfluidic platforms that can mimic large vessels and heart valves. To address this technological gap, a bilayer membrane microfluidic device has been developed to simulate the 3D environment of blood vessels and valves. This device can be utilized for drug screening in a physiologically relevant 3D cardiovascular microenvironment.[49] To further enhance the reproducibility of the in vivo environment, a novel method has been established to culture 3D tissues in combination with a built-in pourable vascular network of a microfabrication scaffold for in vitro OOC drug testing and in vivo surgical vascular anastomosis.[50] The structure, function, and physiological correlation of the 3D cardiac tissue formed in the microfluidic chip are superior to those of traditional two-dimensional (2D) monolayer culture analysis.[51] In conclusion, cardiac chips have become an integral part of drug screening and cardiac disease modeling. They bridge the gap between traditional 2D methods and the real human body, making them more suitable for human cardiac biology and medical research.[44]

Other Vascularized OOCs. Other vascularized OOCs also have been a significant focus in recent years. For instance, the vascularized bone marrow chip has successfully replicated the functions of bone marrow perivascular and endosteal niches in vitro, allowing for the study of drug reactions and interactions with cancer.[52] Additionally, the kidney-on-a-chip has demonstrated the ability to regulate the vascular differentiation of kidney organoids and has shown increased sensitivity to nephrotoxic drugs when cultured in a microfluidic system. It also demonstrated that physiological flow plays an important role in maintaining the physiological function of some kidney organoids.[53] Furthermore, the human-neurovascular-unit-on-a-chip serves as an innovative model for studying infectious human brain diseases and evaluating drug delivery systems targeting the blood-brain barrier (BBB) brain tissues.[54]

The tumor vascular system plays a crucial role in the tumor microenvironment by facilitating the transport of nutrients, oxygen, therapeutic drugs, and immune cells to the tumor site through blood vessels. Therefore, it is imperative to develop a vascularized tumor spheroid model.[55] Microfluidic chips cocultured with cancer cells and endothelial vessels have great potential for studying the interaction between blood cells and cancer cells and for obtaining functional insights and preclinical data on various antimetastatic drugs.[56] Although relevant models have been previously developed, the vascularization of tumor spheroids in vitro generally lags behind that of vascularized tumor tissues in vivo.[57] To improve tumor vascularization, researchers have introduced a novel approach to form tumor spheroids by sequentially adding fibroblasts to preformed tumor spheroids. They have demonstrated that this vascularized sequentially cultured tumor spheroid model can be used to improve drug delivery and cell transport as an in vitro model for studying drug transport kinetics, cell transport, and CAR-T cell response in various tumor cell lines, including renal, lung, and ovarian cancers.[55] Concurrently, lymphangiogenesis and angiogenesis around the tumor spheroid can be effectively induced by promoting interstitial flow. Compared to spheroids consisting of only cancer cells, the vascularized spheroids exhibit stronger uniformity and enhanced angiogenesis around the tumor.[58]

Research indicates that microfluidic vascular systems can be used to evaluate potential anticancer drugs. It has been established that integrin $\alpha v\beta 3$ plays a key role in promoting angiogenesis, making it a more accurate model for the tumor vascular system. As a result, $\alpha v\beta 3$ holds promise as a therapeutic target for cancer treatment.[59] Additionally, the vascularized tumor microfluidic chip platform can be utilized to investigate the impact of stromal cells on tumor cell attachment and growth, as well as the effects of tumor cells on blood vessel and mesothelium permeability in early and late metastasis models.[60] To better replicate the interaction between cancer cells and the tumor environment and enhance the prognosis of neuroblastoma, the Michael research team has designed a microvascular neuroblastoma tumor environment chip. This innovative chip is expected to advance our understanding of tumor angiogenesis and metastasis in precision medicine.[61]

The vascularized tumor-on-a-chip has the potential to elucidate the mechanism and mode of action of candidate drugs through transcriptome sequencing technology, offering crucial data to support the development of treatments for colorectal cancer.[62] The convergence of tumor organoid culture, microvascular engineering, and microfluidic technology holds promise for personalized cancer treatment. However, further refinement of materials and design for microvascular tumor-on-chips is necessary. Notably, the widely used polydimethylsiloxane (PDMS) in tumor-on-chips has the drawback of biomolecule adsorption, which may affect the accuracy of drug testing.[63] In the future, integrating the microvascular tumor model with the immune system could enable a more faithful replication of cancer pathophysiology.

IMMUNE SYSTEM-ON-A-CHIP

The field of OOC technology is poised to emerge as a widely embraced platform for conducting human-specific experiments in preclinical research and therapeutic testing.[64] Currently, OOC platforms have played a fundamental role in studying the involvement of the immune system in various diseases. These platforms have been utilized to investigate immune responses

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and activation during inflammation in OOC units representing joints, bone marrow, skin, intestine, and lung.[65] The efficacy of immunotherapeutic drugs, such as immune checkpoint inhibitors, relies on their interaction with the tumor immune microenvironment within the body. A growing research trend involves the coculture of tumor organoids and immune cells to detect drug sensitivity. The complexity of tumor structure has been a major obstacle for drug diffusion into tumor tissues, necessitating the development of more sophisticated models to replicate the heterogeneity and complete microenvironment of tumors in vivo.[66,67] The concepts of "cancer-on-chip" and "immune organ-on-chip" for disease modeling are still in their early stages. Combining these approaches may offer insights for in vitro cancer immunotherapy research, including the infiltration of immune cells into tumors, potentially leading to the development of next-generation cancer treatments.[68]

Epithelial-immune cell. Epithelial cells are present at all the body's borders in contact with the external environment, such as the skin, respiratory tract, lungs, and digestive tract. They form the body's initial defense against pathogen infection and are the first responders to pathogenic infection. Understanding the interaction between epithelial cells and immune cells is crucial for studying anti-infection immunity and postinjury immunity (Figure 1B).[69] Furthermore, a microfluidic chip mimicking the lymph node (LN) microenvironment incorporates key human LN features, offering a platform to study drug applications in a more physiologically relevant microenvironment for downstream immunology research.[70] Moreover, the use of human intestinal organoid models with intestinal-related lymphoid tissue pluripotent stem cells presents an opportunity to investigate food allergies, intestinal infectious diseases, and the development of mucosal vaccines.[71]

In recent research on immunity against viral infections, the microengineered alveolar chip has revealed the crucial role of immune cells in mediating lung injury and exacerbating inflammation.[40] Moreover, the lung microarray has been utilized to study influenza virus and pseudotyped SARA-CoV-2 host−virus interactions and to screen drugs. The lung-on-chip technology facilitates multicell cocultures, tissue−tissue interfaces, circulating cultures, and circulating immune cells, effectively bridging the gap between in vitro models and the physiological pathology of human organs. This model can be used to study host−virus interactions at the organ level, allowing for the simultaneous study of various cellular responses to viruses. Its advantages include low cost and rapid testing of drug candidates, making it a valuable research platform for current COVID-19 pandemic research.[36]

Tumor and Immune Cells. Organoids are frequently used in the precision treatment of tumors to assist in personalized drug selection based on patients' drug sensitivity.[72,73] Tumor organoids cocultured with immune cells can simulate some characteristics of the tumor microenvironment.[74] Solid tumors create an inhibitory environment around their periphery, leading to nutrient depletion, waste accumulation, hypoxia, and pH acidification,[75,76] which can significantly impact the immune system's ability to fight tumor cells. Researchers have simulated the microenvironment of breast cancer solid tumors by mimicking gradients of nutrition, pH, proliferation, and necrosis using an in vitro tumor microfluidic chip platform. They observed how immune cells, particularly natural killer cells, respond to the tumor-induced inhibitory environment.[77] To assess the drug response testing function of the chip, researchers developed a tumor-on-chip that successfully recreated a complex tumor environment containing fibroblastic stroma and immune cells by combining patient-derived organoids (PDOs) and stromal cells, especially pancreatic stellate cells and macrophages (U937). They demonstrated that targeting the stroma significantly enhanced chemotherapeutic effects on cancer cells, confirming the feasibility of the tumor chip device for drug testing. This paves the way for the design of bioassay tools to optimize drug response and advance personalized precision medicine for pancreatic ductal adenocarcinoma (PDA).[78]

The coculture system is commonly used to study the communication between tumor cells and various components of the tumor microenvironment, such as fibroblasts, endothelial cells, immune cells, and microorganisms. However, there is no standardized method for establishing a coculture system.[79] Researchers have developed a standardized platform for high-throughput drug screening and precise treatment of T cells combined with pancreatic tumor organoids. These organoid models can help in studying T-cell infiltration and the cytotoxic effects on tumor cells in an immunosuppressed microenvironment. This advancement is expected to expedite the discovery of effective combinations of epigenetic drugs and immune checkpoint blockades for PDA and pave the way for new precision immunotherapy in the future.[80] Furthermore, the use of tumor chips combined with real-time imaging and automatic analysis is a valuable approach for better understanding complex biological mechanisms, including immune-mediated anticancer activity. Similar studies can be employed to evaluate immune checkpoint inhibitors or cell therapies.[81]

MULTIORGAN-ON-A-CHIP

The single-organ system is focused on the precise replication of a single organ and can be utilized to assess a drug's impact on a specific organ. Meanwhile, the multiorgan approach involves two or more organs, such as the intestinal-liver chip, liver-kidney chip, intestinal-brain-axis chip, etc. The ultimate complex form could potentially encompass all human body organs, referred to as the human-on-a-chip (Figure 1C).[82] The multiorgan strategy aims to investigate potential interactions between organs resulting from the exchange of drug metabolites or soluble signaling molecules.[83] The Multi-OOC is the most intricate and least documented type,[84] and microfluidic technology could further interconnect multiple chips, facilitating the analysis of multiorgan interactions and real-time drug monitoring.[85]

Challenges and Developments. When developing multiorgan systems, more intricate engineering designs are typically required compared to single-organ systems, as they demand sophisticated fluid control systems to regulate the transportation and distribution of culture fluid among different organs.[86] The decision to opt for a single-organ or multiorgan system when designing a system-on-a-chip hinges on whether the system can achieve the desired function after it has been physiologically modeled. The level of complexity should be minimized to represent the function of the organ.[87] Single-organ chips prioritize the structural authenticity of individual organs, while multiorgan chips emphasize the interaction between different organs.[88] Currently, the main challenge for multiorgan systems lies in achieving acceptable reproducibility and physiological correlates. Key areas to address include the development of blood substitutes and control of the incubation environment, minimizing and characterizing drug adsorption and absorption of platform materials, and integrating physiological considerations into system design.[89,90]

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In 2019, the MINERVA project, which was funded by the European Research Council, made a significant breakthrough in developing the first multiorgan chip platform for microbiota-gut-brain axis engineering. This groundbreaking platform was designed to evaluate the effects of intestinal microflora on neurodegeneration.[91] The MINERVA project faced a major challenge due to the intricate complexity involved in various biochemical pathways, multiorgan functions, and crosstalk.[92] To address this challenge, the researchers developed a four-organ chip that integrates miniaturized human intestines, liver, brain, and kidney into a microphysiological system.[93] This allowed for autologous coculture crosstalk analysis, disease induction, and subsequent drug testing. Furthermore, the project clarified several general design and operating principles for implementing the "physiome-on-a-chip" method of microphysiological systems (MPSs) in drug discovery. Based on this system, platforms such as 7-MPS and 10-MPS have been developed.[94]

Applications of Multi-OOC. Multiorgan chips offer a more comprehensive assessment of drug efficacy and potential toxicity than single-organ chips.[95,96] These chips enable the simultaneous study of drug effects on multiple organs, particularly in predicting liver and cardiotoxicity during preclinical trials. Researchers have developed a liver-heart microarray that can predict off-target cardiotoxicity resulting from hepatic metabolism.[97] In a study on doxorubicin (DOX)-induced cardiotoxicity, liver-heart chips proved more effective in assessing toxicity than traditional static 3D cultures, thus promising to improve preclinical studies.[98] Additionally, liver-heart chips can be used to evaluate the cardiac safety of antidepressants after hepatic metabolism in vitro.[99] Furthermore, a liver-lung chip demonstrated the ability to metabolize compounds and modulate their toxicity.[100] Researchers have developed a liver-brain chip integrated coculture system to assess the liver metabolism-dependent cytotoxicity of antibrain tumor drugs in vitro.[101] This innovative work has led to the creation of a bionic liver-brain model that mimics physiological and pharmacological processes. It provides a straightforward platform for long-term cell coculture, drug delivery, and metabolism, as well as real-time analysis of drugs' impact on the brain.[102]

Multiorgan chips play a crucial role in studying the interactions and crosstalk between different organs. Compared to single-organ cultures, they offer a more comprehensive approach to evaluating the safety and effectiveness of compounds.[103] Researchers have shown the feasibility of 3- and 6-organoids systems, each capable of functioning in a common recirculation medium for at least 21 days. When testing the functional drug response using capecitabine and isoflurane, it was observed that these drugs are metabolized into products with downstream toxicity in other organoids (heart, lung, or brain tissues, depending on the specific drug) when the liver is exposed to them. This indicates that multiorgan chip systems can effectively characterize drug interactions in vitro.[104] In further research, a system comprising eight vascularized dual-channel organoid chips (intestine, liver, kidney, heart, lung, skin, BBB, and brain) maintained activity and organ-specific function for 3 weeks when intermittently fluidly coupled in culture by a common blood substitute through the medium and inner cortex vascular channels.[105] Multi−OOC systems significantly advance research in pharmacokinetics (PK) and pharmacodynamics (PD) and are pivotal for new drug development. Researchers successfully integrated 10 human organoid chips into an automated system to simulate the physiological PK models of drug absorption, metabolism, and excretion in humans for the first time. This demonstrates the capability of the multiorgan chip system to absorb, metabolize, and model drugs, and predict the dynamic changes in drug concentrations in the blood quantitatively, as observed in human clinical trials.[106]

Multiorgan chips play a crucial role in determining the comparative effectiveness of different treatment approaches. Researchers have developed a microphysiological system involving lung, liver, and breast cancer to simulate the effects of inhaling and intravenous administration of curcumin as a model drug. This marks the first successful demonstration of constructing a multiorgan microphysiological system with recirculation flow.[107] The first oral bioavailability parameter assessment system for pharmacological and toxicological applications has been developed to simulate the processes of drug digestion and absorption in the oral, gastric, and intestinal tracts. This integrated gastrointestinal system, with its compartmentalized design, has significantly contributed to the fields of pharmacology, toxicology, and nutrition. In the future, it has the potential to substantially reduce the need for animal models in these areas.[108,109] In the quest to achieve greater biomimicry, researchers have devised chip-based human liver-intestinal and liver-skin coculture systems that mimic the human vascular system. They have successfully simulated the oral route of administration using liver-intestinal cocultures and the systemic route of administration using endothelialized liver-skin cocultures through repetitive administration of the chip-based cocultures (Troglitazone).[110] Researchers have successfully created a multiorgan chip that mimics systemic physiology and diseases by integrating human heart, liver, bone, and skin tissues. This innovative chip replicates the PK and pharmacological characteristics of DOX in the human body and can detect early miRNA biomarkers of cardiac toxicity. Compared to isolated cultured tissues and tissues with fluid interconnection but without an endothelial barrier, the multiorgan chip demonstrated improved predictive accuracy of clinically observed miRNA responses, which has significant implications for its clinical application.[111] Additionally, a dynamic multiorgan chip has been developed to simulate type 2 diabetes and visceral adipose tissues in a hyperglycemic environment. The drug treatment pattern observed on the chip closely mirrored that of patients in clinical trials, further underscoring the potential clinical applications of multiorgan chips.[112]

SENSORS INTEGRATED IN OOCs

The integrated platform allows for real-time biomechanical, biophysical, and biochemical monitoring and characterization, thereby enabling more precise 3D evaluation of organoid structures.[113] Biosensing technology has made advancements in integrating noninvasive technologies and multiorgan systems. This can be achieved by integrating the biosensor into the main platform system or by developing it as a stand-alone module.[89] Organoid sensors can be categorized into mechanical sensors, electrochemical sensors, and optical sensors based on the characteristics of target signals and sensing principles (Figure 2).[114]

MECHANICAL SENSORS

The movement in the lungs, blood vessel shearing, intestinal tract peristalsis, and skin tension are all crucial for the development and function of organoids. Currently, there is no integrated microsystem capable of replicating the complex physiological functions of living organs by integrating multiple

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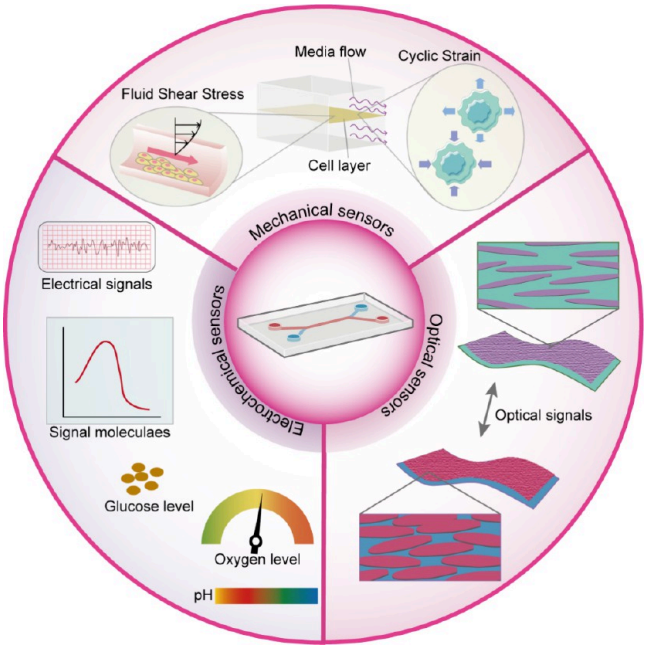

Figure 2. Fundamentals of sensors integrated in OOCs. Sensors are classified based on the characteristics of the target signal and the sensing principle as mechanical, electrochemical, or optical.

tissues and placing them in dynamic and mechanically relevant organ-specific microenvironments.[39] The microfluidic chip serves as a platform for quantifying stress, electrophysiology, and cellular structure.[115] The rapid progress of organoid chips is due to the combination of cell biology, microengineering, and tissue engineering. Engineering techniques that simulate the specific characteristics of tissue microenvironments include shear stress and cyclic strain.[116] Researchers have successfully developed an intestinal organoid chip that, within 5 days, controls fluid flow at the lateral basement to regenerate functional intestinal microstructures. This is achieved with physiological shear stress and mechanical movement (Figure 3A).[117]

The development of microdevices for drug screening, biomechanical analysis, and cardiac physiological studies has been a focus of research. However, existing devices have limitations. Some were highly scalable but only suitable for small molecule testing, lacking the ability to stress tissues.[118] Others could analyze biomechanics comprehensively but were limited to culturing only one tissue, requiring design changes for testing multiple parameters.[119] A new microfluidic platform has been developed to address these limitations. This platform can simulate increased mechanical stress and study treatment schemes leading to cardiac hypertrophy in a high-throughput manner. A pneumatic microfluidic platform has been created to provide stable and repeatable mechanical stimulation of cardiac microtissues. This system allows real-time analysis of tissue phenotype, enabling the study of the effects of mechanical stress on myocardial hypertrophy, simultaneous damage, drug treatment of cardiac tissues, and more rigorous drug testing.[120] Furthermore, in the field of multiorgan chips, researchers have developed a liver-heart chip that enables the physiological beating of microtissues through mechanical training and continuous evaluation of electrical activity (Figure 3B).[97]

The current primary method for tracking the pulsation of heart tissue is optical imaging. It is capable of recording the convulsive movements of heart tissue or generating a Ca^{2+} flux map after fluorescence dye staining. However, it is unable to directly measure the mechanical properties. To tackle this limitation, researchers have developed a flexible electronic skin platform based on nanocrack film for noninvasive dynamic monitoring of heart organoids. The system can promptly identify key mechanical characteristics, such as beating frequency, intensity, and regularity of beating pattern, during the contraction/relaxation process of cardiac organoids in complex microenvironments, including electrical stimulation, electrical resuscitation, drug dosage, tissue culture, and disease modeling (Figure 3C).[121]

Researchers have recently created a microfluidic device to monitor contraction stress in real-time. This device involves wrapping mouse cardiomyocytes in degradable gelatin methacrylate hydrogel, which is then sandwiched between two polyacrylamide hydrogels. The polyacrylamide hydrogel acts as a "stress sensor," and adrenaline is used to enhance the amplitude and frequency of cardiac contraction. This enables the measurement of changes in contraction stress produced by beating heart cells.[122] Additionally, researchers have expanded the applications of hydrogels by developing HiPSC-CM fibers with aligned cardiomyocytes.[123] By culturing cardiomyocytes in hydrogel structures with fixed edges using HiPSC-CMs, they were able to accurately measure the contraction force generated along the direction of the fibers. This method has been used to investigate the change in contraction frequency and contraction force following isoproterenol and cardioplegic therapy, proving to be a valuable tool for analyzing PK in drug development.[124]

Researchers also utilized HiPSC-CMs to create a novel cardiac microarray. This microarray included HiPSC-CMs and 3D cardiac microtissues on a microfluidic chip based on a microelectromechanical system. This setup enabled the visualization of cardiac microtissue pulsation by monitoring particle displacement and the quantification of physiological parameters such as fluid output, pressure, and force. The model exhibited a pharmacological response to isoprenaline, showing a strong correlation between heart rate and particle displacement (Figure 3D).[125] Additionally, a team recently developed a miniature heart chamber replica using nanoengineered components and human heart tissue. This replica allowed for scaled modeling of cardiac mechanical function at the organ level, and the integrated microfluidic system reproduced ventricular flow control function with a complete isovolumetric phase pressure–volume circuit. This advancement suggests that the development of structurally and biomechanically complex tissue systems on an OOC will be crucial for the application of high-precision manufacturing (Figure 3E).[126]

ELECTROCHEMICAL SENSORS

Electrochemical sensors are the most commonly used biosensors for monitoring biochemical parameters such as pH, oxygen, and glucose.[127] Qualitative or quantitative analysis of organoid effluents has provided valuable data on functional biomarkers of tissue health and injury, apparent permeability (Papp), cytokine release, and metabolite and exosome secretion.[105,108] OOC technology can integrate sensors to observe tissue conditions in real time and monitor cell interactions dynamically during experiments. These sensors rely on electrochemical or photochemical reactions, which are difficult to replicate in animal models.[44]

Within the modular OOC integration platform, the OOC platform was housed in a custom benchtop incubator that could

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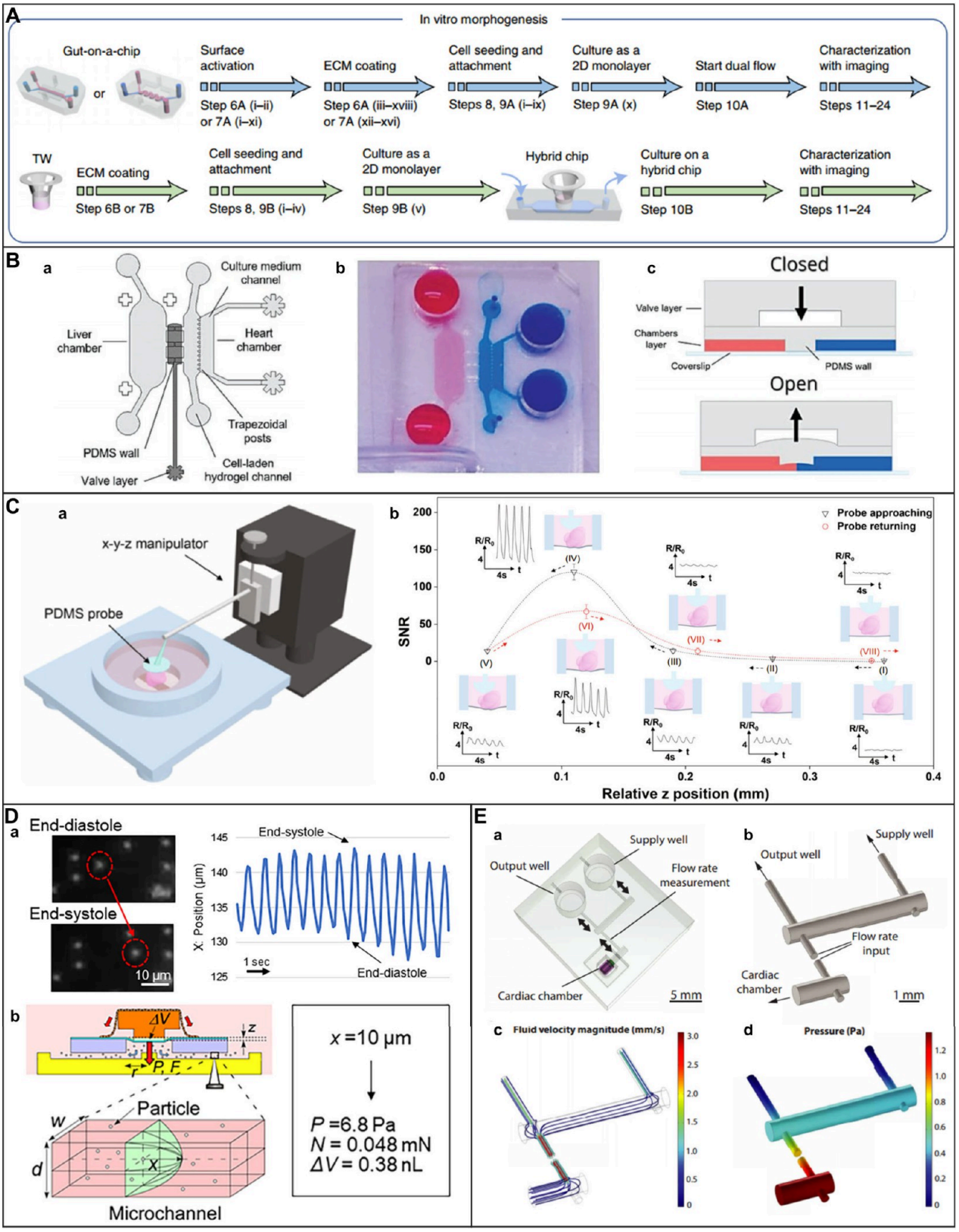

Figure 3. Mechanical sensors integrated with OOCs. (A) Overall steps of Caco-2 or organoid-derived epithelial cell culture on a gut-on-a-chip or on the Transwell insert of a hybrid chip followed by the induction of 3D morphogenesis and characterization of the formed epithelial structure. Reproduced from [117]. Copyright [2022] Springer Nature. (B) (a) Layout of the Valve platform where the liver and heart chambers are separated by PDMS walls. (b) The fabricated Valve platform where the liver and heart chamber are respectively highlighted in pink and blue. (c) Communication between the liver and heart compartments can be achieved by applying negative pressure to the valve layer. Reproduced from [97]. Copyright [2023] Wiley-VCH GmbH. (C) (a) Schematic of the experiment setup of instantaneous organoid beating monitoring by the Pt-based force-sensing diaphragm with soft PDMS probe and *x-y-z* manipulator. (b) Plot of load versus signal-to-noise ratio, overload could impede the contractility of cardiac

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Figure 3. continued

organoid. Reproduced from [121]. Copyright [2022] Springer Nature. (D) (a) Representative pattern of synchronized particle displacement based on beating time of 3D cardiac microtissues. (b) Calculation of the physiological parameters. ΔV = stroke volume, z = vertical displacement of the diaphragm, P = applied pressure, F = applied force, r = radius of the chamber, w = width of the microchannel, d = depth of the microchannel, and x = particle displacement distance. Reproduced from [125]. Copyright [2020] Springer Nature. (E) Using particle image velocimetry to evaluate the pump function of a helical cardiac chamber. (a) A miniPUMP system featuring a beating cardiac chamber and bidirectional chamber-induced flow. (b) Design of the microfluidic simulation model of the device in (a). The flow rate measured in (a) was used as input to the model. (c and d) Examples of the velocity (c) and pressure (d) output of the microfluidic model. Reproduced from [126]. Copyright [2022] American Association for the Advancement of Science.

maintain optimal temperature and carbon dioxide levels. It was equipped with biophysical sensors to detect pH, oxygen levels, and temperature, as well as electrochemical immunomonitoring for protein markers such as glutathione S-transferase, creatine kinase MB (CK-MB), and organoid morphology. Additionally, portable optical microscopes were utilized for real-time monitoring of the effects of common drugs like acetaminophen and DOX on human liver cancer tissues, as well as the impact on normal myocardial tissues, including liver-heart chip and liver cancer-heart chip systems.[85]

Existing methods for monitoring chemotherapy-induced cardiotoxicity (CIC) and model systems developed through in vivo or in vitro CIC platforms often fail to notice early signs of CIC. To address this, researchers developed a heart-breast cancer chip platform to achieve stable, continuous, and repeatable measurement of biomarkers. The sensing platform demonstrated outstanding accuracy, high sensitivity, and an extremely low detection limit. For instance, the sensor could detect cellular biomarkers as low as 0.1 pg mL^{-1} with sensitivities of ∼0.8, ∼1, and ∼0.9 $(\log(ng/mL))^{-1}$ to troponin T, CK-MB, and HER-2, respectively, whereas the detection limit of a traditional enzyme-linked immunosorbent assay for these three markers is several hundred pg mL^{-1}. Consequently, the platform enabled the detection and prediction of early CIC in individual patients (Figure 4A).[90] In order to study the output signal of the cerebral cortex and its transmission to the spinal cord, researchers developed an engineered cerebrospinal assembly system by coculturing brain organoids and motor neuron spheres. Caffeine was used as a model neurochemical to evaluate neural signal transmission. This system effectively monitored the output signal of the cerebral cortex and its transmission to the spinal cord, suggesting that the platform could be used as a screening tool to confirm the transmission of stimulation signals by neurochemicals (Figure 4B).[128]

Integrating microsensors into OOC systems helps cancer research and drug development. Researchers have designed a breast cancer chip that combines microsensors and microfluidics, utilizing electrochemical sensors to monitor cellular oxygen and glucose consumption as well as lactic acid production over a one-week period to observe the effect of drugs on cell metabolism (Figure 4C).[129] To replicate the human physiological environment of actual patients and enable hypoxia-induced tumor metastasis and rapid drug screening on a chip, researchers developed a microfluidic chip that connects two organoids—lung cancer and liver—while monitoring the oxygen concentration through the sensors on the chip. (Figure 4D).[130] Advancements in sensing technology have led to continual improvements in the measuring characteristics of biosensors, including sensitivity, specificity, detection threshold, and sample volume.[131] Single-cell impedance cytometry is a marker-free tool. By leveraging single-cell electrophysiology and dielectric model fitting, it can differentiate subpopulations of cells with known biophysical variances, allowing for the quantification of subpopulation proportions and the prediction of their changes under drug exposure. This provides diagnostic insights for exploring new patient-centered therapeutic drugs (Figure 4E).[132]

OPTICAL SENSORS

Optical sensing methods utilize various techniques, such as fluorescent and luminescent markers, to measure metabolite expression and movement.[133] Traditionally, bioinformatic monitoring of OOCs has been conducted off-chip. Resulting in significant time delays. However, one researcher proposed a more direct optical detection method. This method involves the construction of a bionic, multi-OOC integrated platform comprising engineered skeletal muscle and pancreatic cells. These engineered tissues incorporate optical biosensing technology to monitor contraction-induced myokine secretion and its effects on β-cell insulin production in real-time. This continuous monitoring could yield real-time data that would be valuable for studying insulin production and other metabolic processes that may change within minutes (Figure 5A).[134]

Optical sensors offer significant advantages in real-time detection. For instance, researchers have developed a structural color bioactuator by assembling engineered cardiomyocytes on an elliptical topological poly(vinylidene fluoride) inverse opal. They also integrated biohybrid actuators and microfluidics into a visualizable microphysiological OOC system for cardiomyocyte monitoring and drug testing. The elliptical structure of the inverse opal can effectively induce myocardial cell orientation, and the cellular force can be characterized by the color changes or reflected spectral shifts of the vivid structures of the heart on the designed chip. This approach, which combines light genetic control technology with a visually intelligent biological actuator, can ultimately establish a real-time detection and self-feedback system (Figure 5B).[135]

Biosensing technology holds tremendous promise for studying the role of various biomolecules in specific diseases. Researchers have introduced a DNA-based AND-Gated nanosensor for fluorescence imaging of glutathione (GSH) and apurinic/apyrimidinic endonuclease 1 (APE1) in living cells, animals, and organoids. This nanosensor monitors changes in the expression levels of GSH and APE1 in cells, enabling visualization of GSH and APE1 in organoids with specific tumor imaging and accurately replicating the phenotypic and functional characteristics of the original biological specimens.[136] Current in vitro assays used to test the endocrine disruption of compounds are primarily based on 2D thyroid cell culture, which often cannot precisely assess the safety of these compounds. To address this limitation, researchers have developed a thyroid-on-a-chip using polymer membrane carriers, which enables continuous online oxygen measurement of the effluent through optical sensing technology. This

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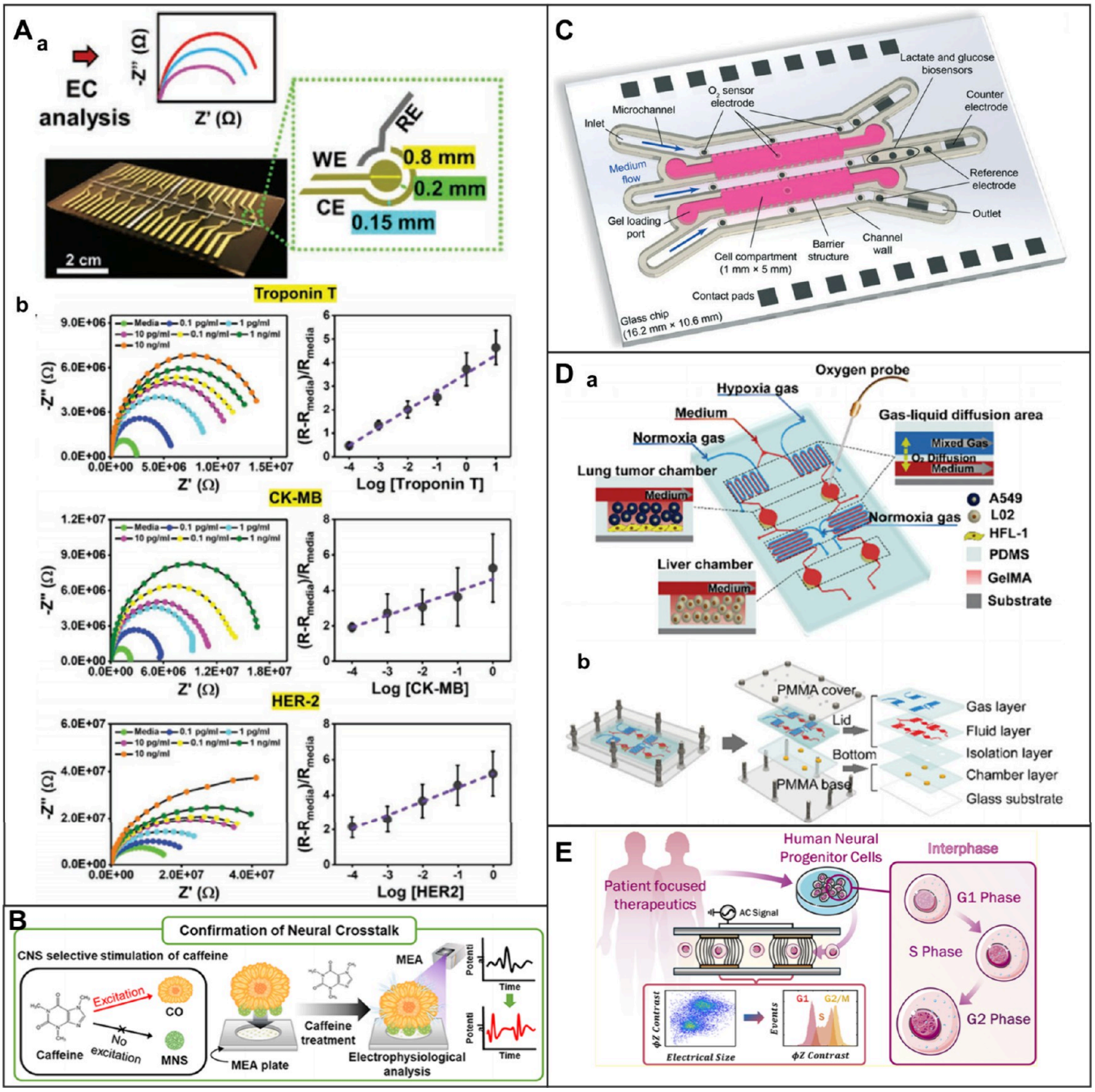

Figure 4. (A) Noninvasive monitoring of biomarkers of cardiac and Breast Cancer spheroids. (a) The sensor chips based on EC impedance spectroscopy measurements for samples from bioreactors. (b) Nyquist and standard curves of multielectrode array chip for representative cardiac biomarkers, Troponin T and CK-MB, and BC biomarker, HER-2 (N = 3). Reproduced from ref [90]. Copyright [2020] Wiley-VCH GmbH. (B) Schematic workflow for the engineered brain-spinal cord assembloid by coculturing cerebral organoids and motor neuron spheroids. Reproduced from ref [128]. Copyright [2022] American Chemical Society. (C) Schematic of the sensor glass chip integrated with electrochemical microsensors for continuous metabolic monitoring. Reproduced from ref [129]. Copyright [2022] Royal Society of Chemistry. (D) Functional Description (a) and multilayer structure (b) of the 3D-culture multiorgan microfluidic platform. Reproduced from ref [130]. Copyright [2021] American Chemical Society. (E) Label-free detection of human neural progenitor cells using high-throughput single-cell impedance cytometry. Reproduced from ref [132]. Copyright [2020] American Chemical Society.

advancement better mimics the structure and function of thyroid follicles and enhances the predictive capacity of such assays. However, the increased complexity of microfluidic chips compared to the current 2D in vitro assays may pose significant technical challenges in large-scale screening (Figure 5C).[137] In order to augment the biological sample size, researchers integrated inverse opal particles into a herringbone mixer, creating a herringbone microfluidic chip. The use of inverse opal particles enhanced the optical sensing capability to respond to the molecular recognition process and report the adsorption state. This platform, characterized by good scalability, reusability, and biocompatibility, holds great promise for clinical blood purification and the construction of artificial kidneys (Figure 5D).[138]

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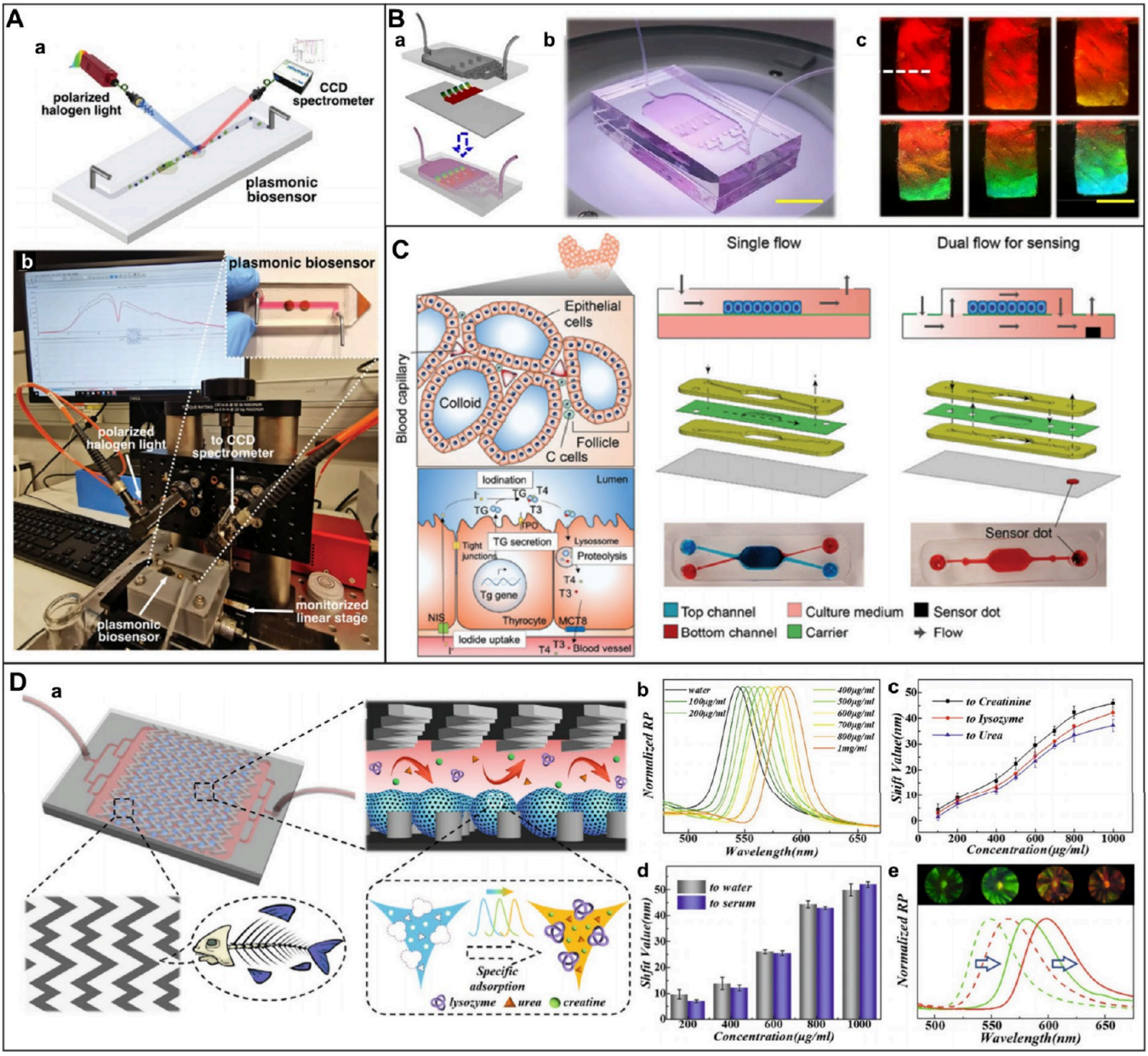

Figure 5. (A) The multiplexed plasmonic biosensor (a) and plasmonic sensing platform (b). Reproduced from [134]. Copyright [2022] Wiley-VCH GmbH. (B) Heart-on-a-chip schematic (a) and photo (b). (c) Optical microscopy images of varying structural colors of a biohybrid actuator in a heart-on-a-chip system during one myocardial cycle. Scale bars, 1 cm in (b), 0.5 mm in (c). Reproduced from ref [135]. Copyright [2018] American Chemical Society. (C) The native thyroid micromilieu and T4 synthesis (left). Thyroid organoid-on-a-chip device (right). Reproduced from ref [137]. Copyright [2023] Wiley-VCH GmbH. (D) (a) Principle of the hierarchical molecular-imprinted inverse opal particles integrated with a herringbone microfluidic chip for efficient biomolecule cleaning. (b) Optical response of the molecular-imprinted polymer inverse opal particles as a function of the concentrations of the target molecules. Reproduced from ref [138]. Copyright [2020] Wiley-VCH GmbH.

■ ORGANOID IMAGING

Many researchers are currently focused on developing new organoids, but they are facing challenges in determining the specific requirements, such as size, shape, and gene expression, that must be met.[139] To analyze the morphology of organoid structures, a combination of microscopic techniques, including optical, confocal, and electron microscopes, should be used. These techniques enable detailed analysis of both the overall structure of the organoids and the individual cells and tissue structures.[84] Researchers can use various laboratory imaging devices to assess cell morphology, marker expression, and cell migration behaviors. The OOC technology allows for in situ immunofluorescence, and fluorescent antibodies and markers can be directly incubated with cells in the chip, eliminating the need for a complex slicing and sectioning process and saving significant time and resources.[140]

■ CHALLENGES IN ORGANOID IMAGING

The imaging of organoids poses several significant challenges, including variations in density and morphology, issues with focus, stitching artifacts in high-throughput images, and the dynamic nature of organoid position and morphology over time. Additionally, the limitations of global analysis of single-frame organoid images further complicate the understanding of growth

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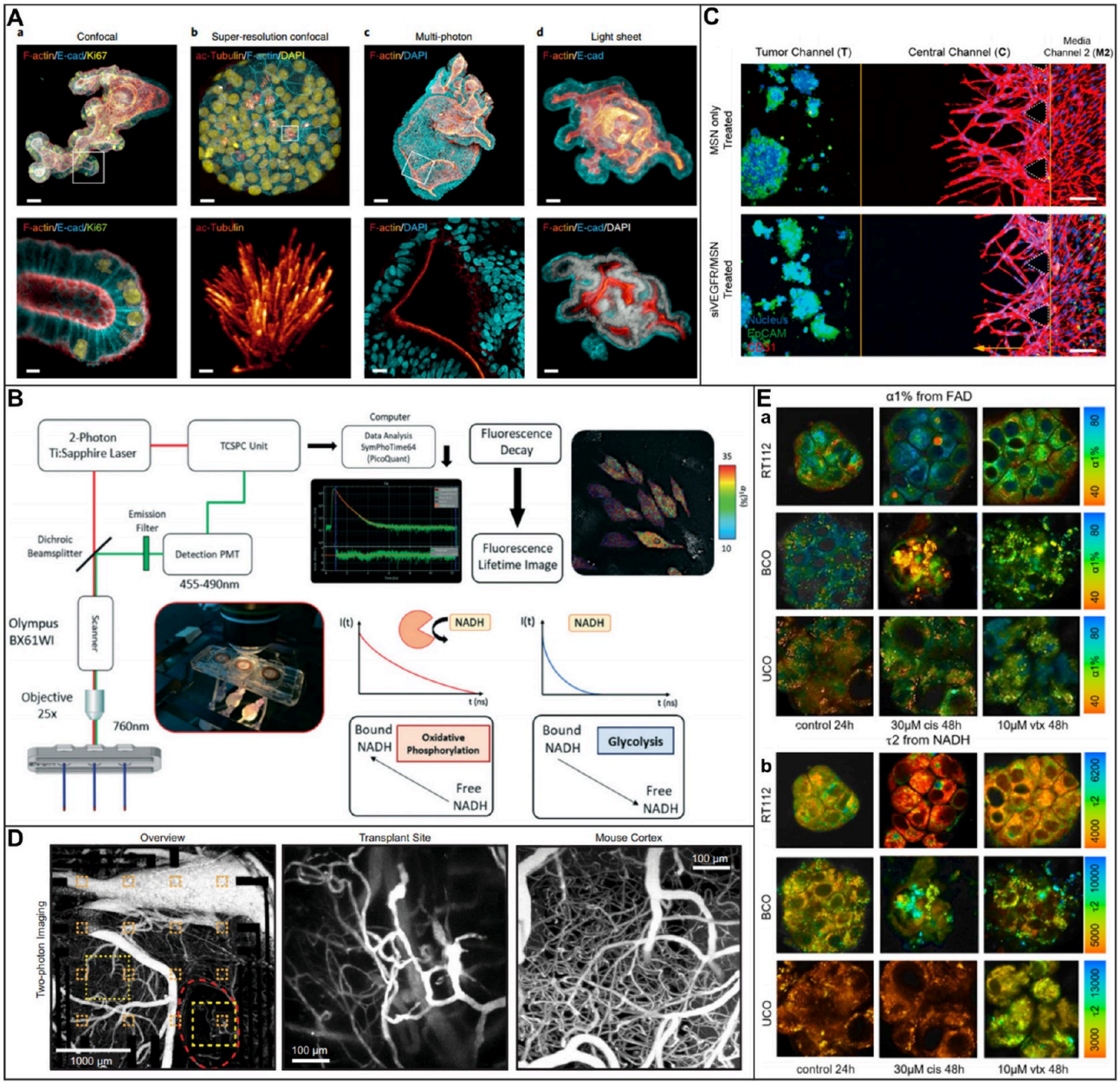

Figure 6. (A) Representative 3D whole-mount organoid images obtained with different light microscopy technologies. (a) Whole-mount 3D confocal image (top) and enlarged optical section (bottom) of a human colonic organoid immunolabeled (fructose-glycerol clearing; 25× oil objective). Scale bars, 20 μm (top) and 5 μm (bottom). (b) The Airyscan Fast module was used to obtain a confocal 3D whole-mount image (top) and enlarged area (bottom) of a human airway organoid (40× water objective). Scale bars, 15 μm (top) and 2 μm (bottom). (c) Multiphoton 3D whole-mount image (top) and enlarged optical section (bottom) of a human colonic organoid (no clearing; 32× water objective). Scale bars, 50 μm (top) and 10 μm (bottom). (d) Light-sheet 3D whole-mount image (top) and optical section (bottom) of a human colonic organoid (fructose-glycerol clearing; 20× objective). Scale bars, 15 μm. a−c were rendered in Imaris using the transparent 3D mode (MIP mode), and d was rendered in Imaris using the blend 3D mode. Reproduced from ref [145]. Copyright [2019] Springer Nature. (B) The process of noninvasive measurement of cellular metabolism of MSCs cultured inside a miniaturized optically accessible bioreactor. Reproduced from ref [147]. Copyright [2021] Royal Society of Chemistry. (C) Representative confocal 3D image in orthogonal view of human hepatocellular carcinoma cells (HepG2) angiogenesis assay, Scale bar, 200 μm. Reproduced from ref [148]. Copyright [2020] American Chemical Society. (D) The representative result from two-photon imaging. Reproduced from ref [149]. Copyright [2022] Springer Nature. (E) The representative FLIM images of bladder cancer cell line RT112, bladder cancer organoids (BCO), and urine-derived organoids (UCO) with flavin adenine dinucleotide (FAD) α1% (a) and nicotinamide adenine dinucleotide (NADH) π2 (b), Scale bar, 25 μm. Reproduced from ref [150]. Copyright [2022] MDPI.

characteristics and morphological changes during culture and drug administration.[141]

Fluorescence lifetime imaging (FLIM) encompasses a range of methods for measuring the luminescence lifetime of various exogenous and endogenous luminescent molecules. Over the years, various techniques for measuring fluorescence and phosphorescence decay have been developed and applied to the metabolic imaging of endogenous and exogenous metabolic markers.[142] Traditional wide-field fluorescence microscopes suffer from low resolution and lack optical slicing capability, making them unsuitable for 3D organoid imaging. Laser scanning confocal microscopy (LSCM) has become the most

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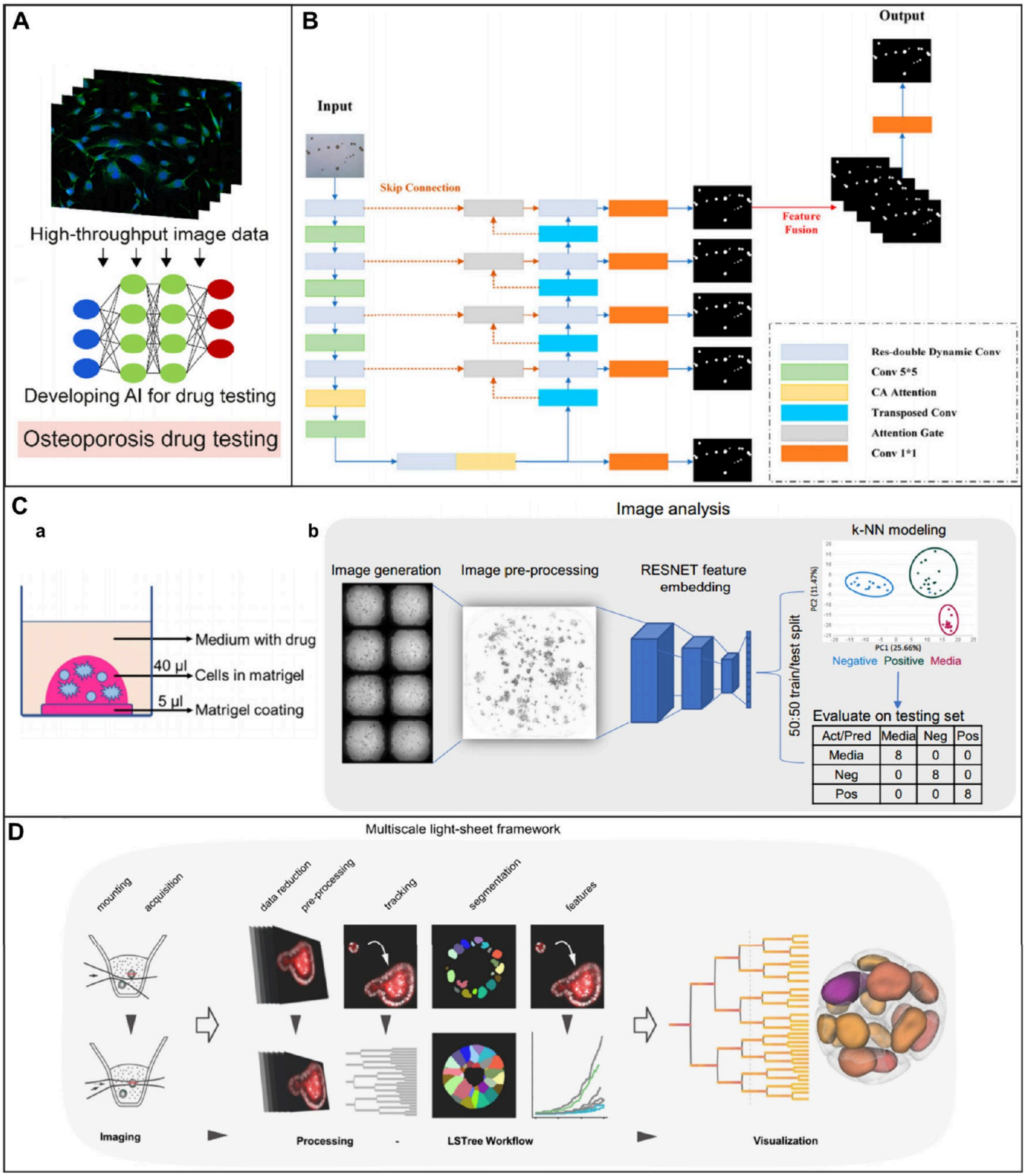

Figure 7. (A) Osteoporosis drug testing and image data analysis using deep learning algorithms. Reproduced from ref [155]. Copyright [2022] Wiley-Blackwell. (B) The RDAU-Net model structure. Reproduced from ref [161]. Copyright [2022] Frontiers Media S.A. (C) An analytical workflow to map visually similar wells (phenomimetics) to reference control wells using an image-embedding approach to convert images into a descriptive numeric vector. The resulting k-NN model then maps the drug-treated well statistics to the reference control. Reproduced from ref [162]. Copyright [2021] Springer Nature. (D) A multiscale imaging framework including acquisition, preprocessing, automatic tracking, segmentation for further feature extraction, and visualization dedicated to 3D live imaging. Reproduced from ref [164]. Copyright [2022] Springer Nature.

widely used method for organoid imaging. LCSM generates optical slices by scanning the sample point by point with a focused laser beam and utilizing a pinhole to exclude defocused background fluorescence, allowing for specific slice and 3D imaging with a penetration depth of ~100 μm. However, LSCM and two-photon microscopy have drawbacks such as low image

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acquisition rates, photobleaching, and phototoxicity, which limit their long-term imaging capabilities. Notably, the combined use of multiple imaging techniques has the potential to compensate for individual deficiencies and maximize imaging efficiency.[143]

The integration of OOC technology with biological imaging has proven to be a promising approach. Achieving high-quality imaging in biological systems necessitates addressing challenges such as high resolution, a large field of view, deep penetration, and high speed. However, OOCs often pose limitations in terms of light penetration and exhibit significant light scattering due to their high cell density and large size.[127,144] To address the issue of limited light penetration in 3D imaging of organoids, researchers have employed transparency technology. A solvent-based, high refractive index matching technique utilizing a fructose-glycerol-based organoid transparency method has been developed. This method offers the advantages of shorter processing times and enhanced safety. Experimental validation of this approach has been conducted on various organoids, including the airway, colon, kidneys, liver, breast cancer, and mouse mammary organoids (Figure 6A).[145]

Moreover, the transparent nature and continuous perfusion of most microfluidic platforms enable good optical accessibility and time-resolved sampling of the perfusing medium. However, these methods have limitations, particularly in real-time monitoring.[146] Consequently, investigators have developed and validated a miniaturized platform for metabolic profiling of mesenchymal stem cells on a chip. This platform mimics the microenvironmental conditions of perivascular ecological niches and facilitates high-resolution imaging of cellular metabolism. It allows for the assessment of oxygen tension gradients and real-time monitoring of cellular metabolism, thereby providing a reliable tool for disease modeling and drug screening (Figure 6B).[147]

Furthermore, the visualization of tumor vasculature in three dimensions is crucial for the accurate evaluation of antiangiogenic nanodrugs based on RNA interference, although it remains a challenging task. In response to this, researchers have integrated 3D microfluidic tumor angiogenesis models with 3D image analysis technology to achieve visualization of the 3D tumor angiogenesis process. This integration offers a reliable strategy for preclinical evaluation of the antiangiogenic efficacy of nanodrugs (Figure 6C).[148] Additionally, two-photon imaging has been utilized to visualize the blood vessels of organoids. Combined with transparent microelectrode arrays, longitudinal and multimode monitoring has been conducted for human cortical organoids transplanted into the posterior cortex of adult mice. This approach has facilitated the visualization of the vascularization of transplanted organoids (Figure 6D).[149]

When using the immunofluorescence technique for imaging organoids, challenges such as a weak fluorescent antibody signal, a faint signal displaying immuno-labeled nucleoprotein, and prolonged sample fixation time may arise. Additionally, once the organoid is processed, it can no longer be utilized for therapeutic purposes, thereby restricting the exploration of alternative organoid-related therapies. There is a growing trend in the development of imaging techniques that do not rely on fluorescent labeling and can be used for long-term tracking.[143] A labeling-free imaging approach, which uses Raman microscopy (RMS) and FLIM, has been established for in situ cellular analysis and metabolic monitoring during drug therapy. Furthermore, FLIM and RMS enable noninvasive and molecularly sensitive tumor-drug interactions, offering the potential to identify and optimize patient-specific therapeutic effects (Figure 7e).[150] Mass spectrometry imaging has also emerged as an important tool for metabolic microscopy, providing information on the label-free, untargeted molecular and spatial distribution of metabolites in tissues.[151] In the study of multiorgan systems, researchers have developed fluorescence microscopy modules to extend the system's analytical capabilities, stand-alone microimaging modules to capture organ-driven static images and dynamic processes, customized software systems to facilitate a complete graphical programming approach with advanced functionality such as error correction, and networked systems for remote experimental design, operational monitoring, and software updates.[105]

■ ORGANOID IMAGING BASED ON AI

The imaging and analysis of OOC systems pose a significant challenge due to the intricate nature of the chip's internal engineering tissue structure. However, AI has shown promising results in medical image analysis.[140] AI aims to enable computer systems to perform tasks that typically necessitate human intelligence. Through training on extensive data sets, AI can make predictions, classify objects, and execute complex tasks.[152,153] Machine learning (ML) is a field within AI that emulates or replicates human learning activities using computers. ML is at the forefront of intelligent research, and AI algorithms can be trained on organoid images to swiftly and accurately analyze the structure and function of organoids.[154] In the realm of FLIM, ML enhances the analysis and computation of fluorescence lifetimes, as well as the clustering and segmentation of image species. Moreover, ML can aid in labeling and even predicting data when integrated with other analytical methods. A subset of ML known as "deep learning" employs artificial neural networks to mimic the human brain's learning process.[140] Combining AI-based image analysis with a high-throughput bionic bone graft platform has led to the development of a system capable of detecting the efficacy of a newly developed antibody-based osteogenic drug through β-catenin translocation analysis. Furthermore, the platform's performance is evaluated through AI-based deep-learning analysis, which uses a considerable amount of image data obtained by the platform. This approach has verified the feasibility of using intelligent deep-learning algorithms in osteoporosis drug testing (Figure 7A).[155]

The integration of tumor chips with real-time imaging and automated analysis represents a valuable approach to addressing complex biological mechanisms.[81] Segmenting single cells in microscope images is typically the initial step in many biological image analysis tasks, and it presents a significant computation challenge. Establishing a training set of high-quality data is essential for deep-learning methods. To this end, researchers have developed a 3D-cell-annotator software using the widely employed 3D Interface Medical Imaging Interaction Toolkit, achieving segmentation accuracy comparable to human experts.[156] A novel neural network-based deep-learning detection method for epithelial cells has been introduced. This method can capture subtle differences in the morphology of the epithelial spheres from bright-field images in a noninvasive manner, track changes in the lumen structure of tissue spheres, differentiate between polarized and nonpolarized pulmonary epithelial spheres, and assess drug effects.[157] Researchers have also devised a method for scoring drug-induced phenotypes in a 3D model system using automated microscopy and image analysis. They have applied this method to screen drugs released

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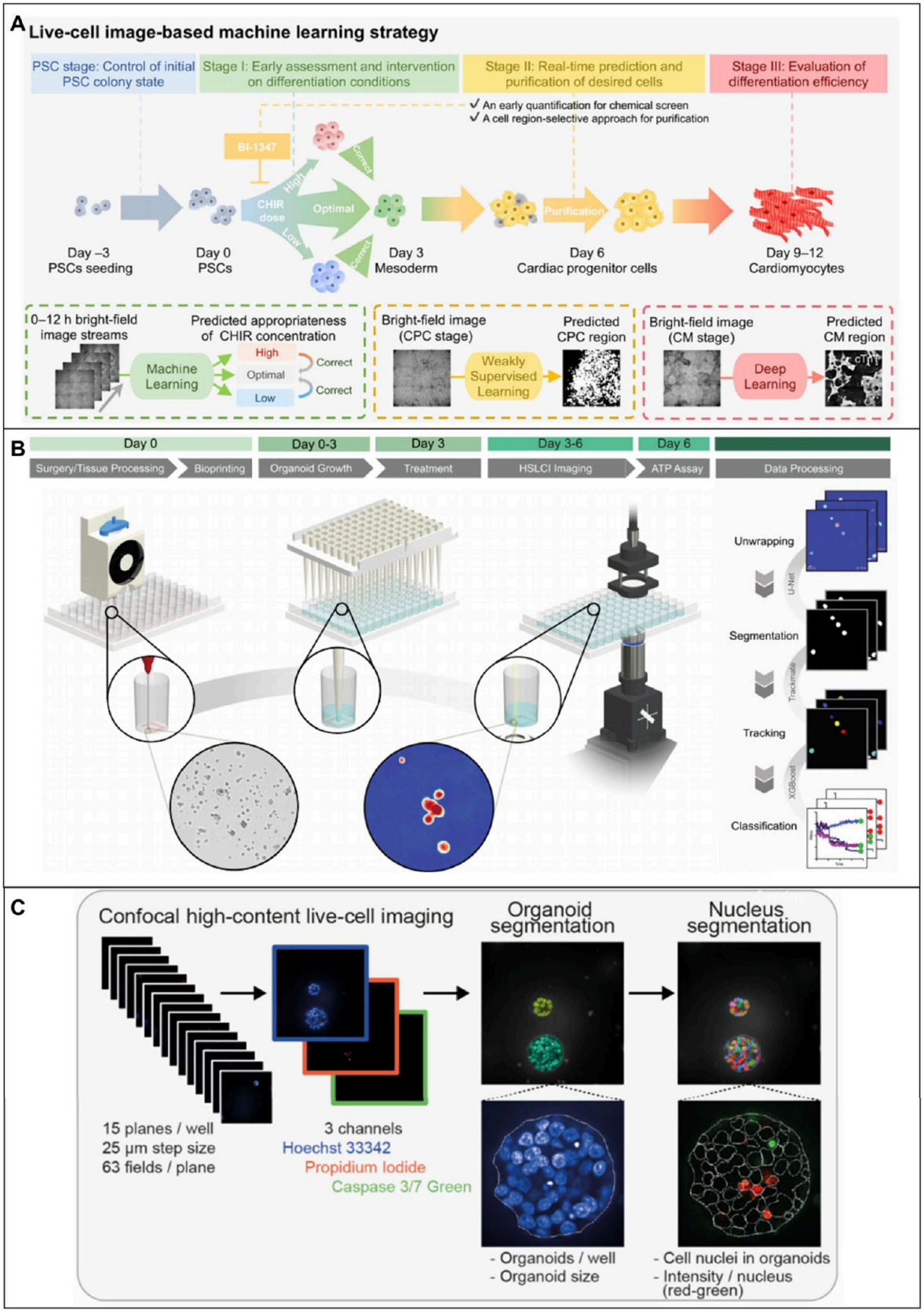

Figure 8. (A) Strategies for using machine learning models based on bright-field images of living cells. Reproduced from ref [167]. Copyright [2023] Springer Nature. (B) Schematic of organoid tracking using HSLCI. Reproduced from ref [168]. Copyright [2023] Springer Nature. (C) Image analysis pipeline for the segmentation and quantification of individual organoids and cell nuclei in organoids. Reproduced from ref [169]. Copyright [2023] MDPI.

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from PDO structures to monitor susceptibility in KRAS mutant colon cancer.[158]

Researchers employed a combination of computer vision and the convolutional neural network (U-Net) ML method to create automated living cell image analysis software. This software was compared to the gold standard CellTiter-Glo 3D analysis, and optimal parameters and drug response indicators were determined to enhance patient stratification.[159] They trained the U-Net using computer-generated biological image data produced by the conditional generative adversarial network to perform semantic segmentation. Additionally, adaptive morphological filtering was utilized to identify organoid instances, and an instance segmentation correction tracking program with shape similarity constraints was employed to reliably select organoid instances of interest.[160] To elevate feature extraction and recovery ability, the res-double dynamic conv attention U-Net (RDAU-Net) model was used. This improved the efficiency and accuracy of organoid drug screening. The researchers implemented a bladder cancer organoid system to segment the organoid in the input image based on the U-Net framework. They then calculated the areas of all the organoids in the image to reflect the growth speed of the organoids, enabling the evaluation of the impact of anticancer drugs (Figure 7B).[161] The first published deep-learning model for organoids introduced a novel deep neural network (dNN) designed to efficiently identify organoids and monitor them throughout cultivation. The approach consists of two main stages. Initially, a series of sequential images were analyzed frame by frame to detect various types of organoids. Subsequently, the similarity between organoids in consecutive frames was computed, enabling the pairing of organoids in adjacent frames, thus achieving rapid and precise detection and tracking of organoids.[141] Epithelial-mesenchymal transition (EMT) is linked to stem cell characteristics and the development of therapeutic resistance in cancer cells. To identify drug candidates capable of reprogramming EMT, researchers have devised a screening technique that leverages universal image features from pretrained dNN in conjunction with a k-nearest neighbors (k-NN) model to establish a similarity comparison between experimental processing and reference control. This method led to the identification of small molecule inhibitors capable of reversing EMT in epigenetic drug screening, potentially offering novel therapeutic approaches (Figure 7C).[162] Researchers have devised an image analysis tool named D-CryptO, based on deep learning, to facilitate the imaging of more intricate organoid structures. This tool automatically assesses the crypt formation and opacity of colorectal organoids from bright-field images, enabling the determination of structural maturity.[163] Gustavo et al. introduced an experimental and image processing framework that utilizes deep-learning technology to optimize imaging and data processing, allowing for the segmentation of individual organoids in 3D form over extended periods. This research provided detailed insights into specific subcellular behaviors across biological scales and enabled the long-term tracking of tissue development, marking an important step toward establishing comprehensive and quantitative digital organoid maps (Figure 7D).[164]

HIGH-THROUGHPUT DRUG SCREENING

High-content cell imaging and analysis technology enables the rapid and automated capture of images of cells, subcellular structures, or tissues in batches, as well as the quantification of cell phenotypes. This technology has advanced rapidly in recent years and is widely used in various areas of biomedical research, including organoids.[165] In an effort to simplify organoid culture and high-content 3D imaging, researchers have introduced an automated multiscale 3D imaging platform. This platform combines high-density organoid culture with fast, real-time 3D single-objective light-slice imaging, providing a user-friendly instrument for simplifying organoid culture and high-content 3D imaging. The platform requires minimal operations and boasts a throughput of 300 organoids per hour. Additionally, it employs deep-learning-based algorithms to quantify the morphology of multiscale organoids, ranging from the subcellular scale to the whole organoid level.[166]

It is necessary to activate Wnt signaling (CHIR99012, CHIR) in order to study myocardial cell differentiation. Researchers utilized deep-learning imaging techniques to identify correctly and incorrectly differentiated cells, observe the entire cell differentiation process, and predict the appropriate drug concentration under bright-field conditions. This noninvasive strategy, based on cell bright-field dynamic images and ML, can determine the optimal state of initial differentiation of pluripotent stem cells in real time and conduct small molecule screening (Figure 8A).[167] High-speed live cell interferometry (HSLCI), a nondestructive imaging method for observing and measuring the weight of living cells in real time, has been combined with bioprinted cells and enabled by ML algorithms. It is capable of accurately measuring the mass of thousands of organoids simultaneously. This information can help determine the therapeutic sensitivity or drug resistance of the organoid and can be used to swiftly select the most effective treatment option for a patient (Figure 8B).[168]

Researchers have developed an imaging-based drug testing program using high-level fluorescence microscopy to explore cellular and drug responses in individual organoids in large-scale tumor drug testing. This program is designed to detect various forms of cell death in living prostate cancer organoids, allowing early and rapid drug testing while preserving tumor heterogeneity within the patient (Figure 8C).[169] Optical metabolic imaging of organoids derived from primary tumors has shown promise in predicting and measuring therapeutic responses in xenografts and human tumor-derived organoids.[170] The development of OOC technology, coupled with advancements in throughput, has generated large amounts of data. AI and deep-learning algorithms are poised to play a crucial role in processing this data, improving OOC design and control, and accelerating phenotypic drug screening. The combination of OOC and AI is expected to provide a powerful tool for future drug efficacy evaluation, particularly for in vitro assessment of drugs with complex chemical systems, and holds great promise for personalized medicine, pharmacology research, and new drug development.[171]

CONCLUSIONS AND PERSPECTIVES

The use of OOC technology presents significant advantages in drug screening. OOCs enhance the bionic properties of cell sources, provide easier standardization and automation compared to traditional cell models, and reduce the impact of human factors, resulting in more consistent and reliable drug response data. Additionally, OOCs offer cost effectiveness and high throughput, making them favorable over in vivo drug efficacy evaluation models.[5] Currently, OOCs are predominantly employed in various stages of drug discovery, including early detection, lead optimization, preclinical evaluation, and preclinical pharmacodynamics and toxicology research.[172]

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In the initial stages of drug discovery, OOCs aid in developing disease models that closely mimic human gene expression and physiological structure, enabling the identification and validation of drug responses. This facilitates a deeper understanding of potential disease mechanisms and the development of more effective treatments. During lead optimization, OOCs provide human-relevant response data, allowing for the optimization of candidate leads with the greatest effectiveness and least toxicity, thus reducing the need for animal testing and promoting safer drug candidates for further development. In preclinical safety evaluation, OOCs serve as screening tools to predict various toxicity mechanisms, especially beneficial for evaluating novel drugs like biological agents and immunotherapy. Finally, in clinical trials, OOCs can be used to study candidate drugs that produce unexpected toxicity signals, providing insights into toxicity mechanisms and guiding the prioritization of candidate drugs.[10]

The past decade has seen rapid development of OOC technology. However, the challenge of a functional vascular system persisted, prompting the creation of various vascularized OOC platforms. This review introduces recent developments in vascularized OOCs, with a focus on liver, lung, heart, and tumor organoids. Currently, the mechanisms and factors promoting vascular system development, including interactions with parenchymal cells, microenvironment suitability, cytokines, and growth factors, are not fully understood. Nonetheless, it is believed that with the discovery of these mechanisms and factors, future induction of blood vessel formation within organoids will be more robust and effective.[18] One potential future direction for OOCs is the interconnection of multiple organs from the organ level to the system level. In a multiorgan system, the construction of blood vessels is essential to more accurately mimic drug reactions in different human organs and to obtain valuable research data guiding clinical research.[82] Progress in the widespread application of OOCs is expected to leap forward in the next 3−5 years.

However, the wider application of OOC technology still faces certain challenges. First, the OOC industry is in the early stages of development, and to achieve widespread use, the future trend is the development of more automated, intelligent, and integrated systems. It is also essential to develop standardized automations of OOC detection instruments.[173] Additionally, qualitative or quantitative analyses of effluents should aim to simplify both the operation and retrieval process, providing functionally relevant data such as biomarkers, Papp, cytokine release, and metabolites. Ideally, OOC devices should be compatible with existing infrastructure, including mainstream laboratory imaging technologies and high-content imaging, combined with AI intelligent analysis and quantitative data analysis.[174] Finally, the materials used in the fabrication of OOCs are crucial. While PDMS is widely used due to its excellent properties of optical clarity, flexibility, and gas permeability, it absorbs small molecules nonspecifically, including certain drugs. Other materials, such as polyurethane, poly(methyl methacrylate), and cycloolefin polymers, are also being developed.[175] The multidisciplinary nature of the OOC industry demands further development to overcome these challenges, thereby facilitating more effective drug screening and bringing the vision of human-on-a-chip closer to reality.

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Y.H. conceived the outline. Y.H. and Q.H. wrote the manuscript. T.L. and Y.W. supervised the manuscript.

Notes

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