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LETTER TO THE EDITOR, NEWS AND VIEWS

Organoid-based 3D in vitro microphysiological systems as alternatives to animal experimentation for preclinical and clinical research

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Editorial/commentary

Animal testing was the only way to assess a drug's safety and efficacy before human clinical trials. To get a therapeutic approved in the United States (US), the Food and Drug Administration (FDA) required toxicity testing on animals like rodents (e.g., mouse or rat) and nonrodents (e.g., monkey or dog). Following the US, countries across the globe likewise adhere to similar regulations. Consequently, millions of animals are held captive in laboratories and cages, awaiting their turn to be utilized in studies by tens of thousands of companies and universities yearly. This increases the ethical concerns and expenses associated with drug discovery research. Although there are well-established guidelines for ethical conduct for the use and care of animals in research, there is always room for improvement (National Academies of Sciences 2021). The lack of public openness in scientific research using animals has also prompted concerns. However, more than nine out of ten therapies that reach human clinical trials fail, owing to safety or effectiveness concerns, which raises the question of whether animal-effective therapies are beneficial for humans and, that too, at what cost (Akhtar 2015). Hence, several alternatives to animal testing were developed to address the lacunae associated with animal experimentation. The recent success of in vitro human-specific 3D models (like organoids and microfluidic-based organs-on-a-chip) that better mimic human physiology and architecture have paved the way for cutting-edge substitutes for animal-based research. Scientists believe that the fast-growing current technologies provide better human-biology-based specific models, improving the lives of people and animals (Horejs 2021). Early accomplishments in the quest to replace animal testing started by outlawing animal-tested cosmetics in different US states. Another significant step forward is the current FDA announcement (FDA Modernization Act 2.0) that new therapeutics no longer require animal testing, according to legislation signed by President Joe Biden in late December 2022. After 80 years of drug safety regulation, this long-awaited step may serve as a critical step in the gradual elimination of animal experimentation.

A resounding collective thinking has emerged where scientists, financiers, and politicians now rely on developing cutting-edge, human-physiomimetic research approaches to spare animals from testing. Astounding advances have also been made with the bioengineering of laboratory-based in vitro human disease-specific models developed using human 3D cell cultures like organoids, spheroids, tumoroids (tumor-like organoids), cell co-cultures, and organ-on-chip technology (Zhao et al. 2022). Microphysiological systems (MPS), the umbrella word for the emerging discipline, encompasses miniature human in vitro organ constructs like organoids, tumoroids, and organs-on-a-chip and can serve as a physiomimetic model, revealing the disease physiology sincerely (van Berlo et al. 2021). Organoids are 3D clusters of stem cell-derived cells that structurally and functionally resemble specific tissues or organs in a dish; organoids of the heart, for instance, beat just like the real thing (Horejs 2021). Organs-on-a-chip are miniature tissues, organoids, or stem cells grown in microfluidic blocks and with microfluidic circuits that activate the physiological mechanics (Leung et al. 2022). Over the previous two decades, scientists and researchers focused on innovating organoid bioengineering protocols and have learned much about organoids and tumoroids and discovered that these non-animal human-specific models might improve disease treatment. Organoid technology can produce tissue-specific

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or disease-specific organoids and provides a unique opportunity to mimic the physiology and complexity of human diseases, and it can also be used for personalized medicine. Considering the variance between human and animal disease pathophysiology, a paradigm shift was necessary to represent human diseases effectively (Zhao et al. 2022).

The 3D human organoid platform is aiding scientists in grasping the pathobiology behind human diseases. Furthermore advances in single-cell RNA-sequencing, epigenomics, proteomics, spatial transcriptomics, spatial multiplex immunofluorescence profiling, CRISPR-Cas-based genome engineering, 3D bioprinting, artificial intelligence-assisted programs, and in silico computer modeling approaches can help improve the comprehensive understanding of these 3D in vitro human-specific models for preclinical and clinical trials (Zhao et al. 2022). The Human Cell Atlas (HCA) is a pioneering global collaboration that uses single-cell technologies to promote scientific research and therapy. HCA project aims to characterize single cells in organoids and other complex in vitro systems using big datasets from human organoids. Current advances in MPS design and assembly using microfluidic chips and onboard microfluidic pumps can help integrate multiple MPS platforms (like lungs, gut, brain, etc.) to study the lung-gut-brain axis in the context of disease development (van Berlo et al. 2021). Similar physiomimetic models can also be used to study interactions between several MPS and the immune system in the context of disease pathogenesis, especially in cancer, metabolic diseases, and rare diseases. Organoid-based 3D MPS can help decipher the role of dynamic in vitro microenvironment on organ development and disease incidence while considering multi-parametric data. The use of organoids during the COVID pandemic was eye-opening and highlighted the importance of organoids and 3D culture systems in faster drug development (Sanyal and Paul 2021). Tumoroids are much more predictive of the efficacy of cancer treatments than animal models. In addition, organoids are effectively utilized to test liver and other organ toxicity, pharmacology, pharmacokinetics, and pharmacodynamics studies but need to be biobanked to support large and long-term studies.

Organoid biobanking entails collecting, storing, and distributing organoid samples for research and therapeutic use. Daily clinical tissue collection yields excess tissue that may be utilized to construct organoid biobanks and provide many future research possibilities (Mukherjee et al. 2022). The different organoid biobanks include tissue-specific, disease-specific, patient-specific (for personalized medicine), and cell type-specific (like adult stem cell, embryonic, and induced pluripotent stem cells) to study stem cell biology and organ development. Several groundbreaking discoveries have been achieved in fundamental and translational biomedical research due to extensive biobank studies of human tissue. Though tissue banks are established, many functional studies cannot be performed on preserved tissue, particularly in translational research. Without using an animal model, we can now comprehend all the physiologically important, disease-associated, human-specific information, circumventing the high cost and long wait period of animal-based experiments, which potentially can be a game changer from a pharmaceutical standpoint. Without validated animal models, 3D human organoid models are critical for modeling rare human diseases (Zhao et al. 2022). Hence many startups are currently operative in this lucrative domain of organoid technology, as several therapeutics have now entered trials based on data produced from organoid or organs-on-a-chip platforms. Though everything sounds promising, several challenges limit the widespread use of these platforms. Figure 1 describes the sequence of events in the generation of organoids and their applications (Fig. 1).

Though promising, the technology has not yet reached the pinnacle, and a total replacement of animal-based experimentations may take decades. Various obstacles, including the lack of robust validation methods, appropriate standards, absence of large-scale production protocols, funding, regulatory rules for stem cell use and organoid commercialization, and ethical issues, riddle the anticipation of a total replacement (Mukherjee et al. 2022). High variability and heterogeneity of the initial starting cells can ruin the possibility of creating uniform organoids. Another critical limitation of the organoid systems is the short amount of time that they can be maintained in culture, subsequent long-term storage, and thawing. Organoid bioengineering also needs further advances in novel biomaterials (like natural and synthetic hydrogels) that mimic the extracellular matrix and cellular microenvironment's physical, mechanical, and biochemical characteristics (Schutgens and Clevers 2020). Lack of vascularization is another critical area that needs attention, and engineering concepts with 3D bioprinting can be a game changer. Current models are reductionist approaches, and modeling complex human diseases in a dish is a real challenge and may need decades to eventuate (Andrews and Kriegstein 2022). Convincing the medical fraternity can also be a critical hurdle that needs to be addressed globally for the acceptance and large-scale use of advanced 3D culture technologies. The goal in the upcoming years should be to design projects focusing on substituting animal-based disease models with appropriate human-cell-based 3D in vitro models by encouraging better research and accessibility. Promisingly, when scientific evidence supports it, several organizations have initiated collaborations with academics, businesses, and regulatory bodies to expedite the implementation of these model systems to come up with a therapeutic solution. FDA has started working with Defense Advanced Research Projects Agency and the National Center for Advancing Translational Sciences to develop MPS-based ecosystems for tangible interventions targeting different

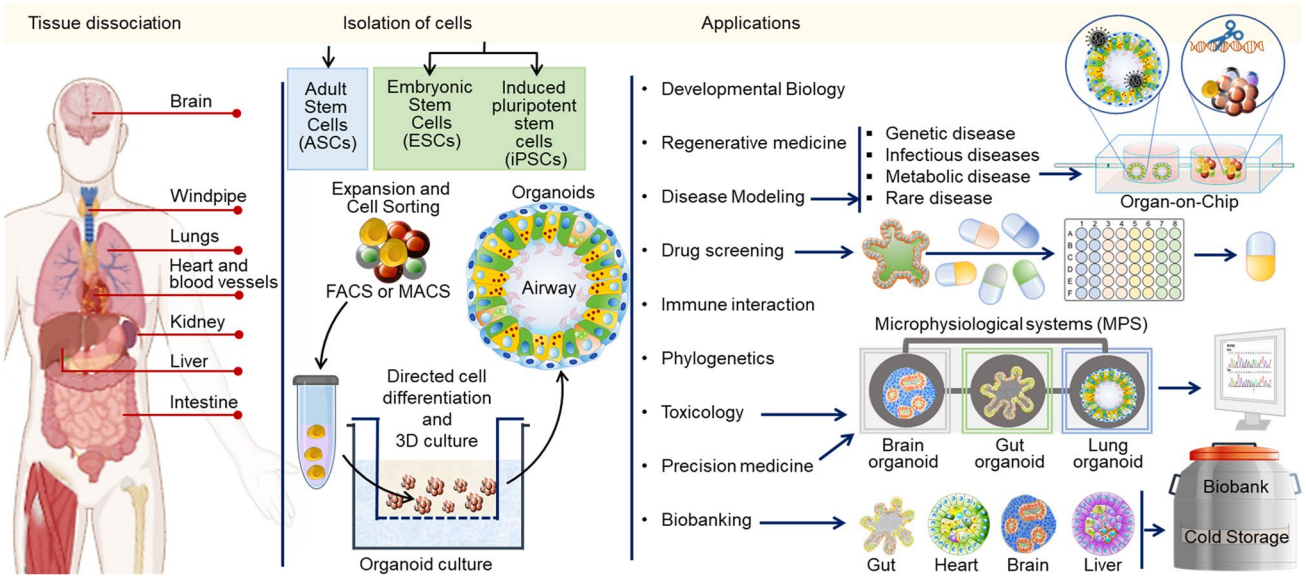

Fig. 1 Modern technologies as alternatives to animal experimentation. Organoid-based 3D in vitro microphysiological systems (MPS) for preclinical and clinical research can serve as alternatives to animal experimentation. Tissue dissociation from several organs, followed by stem/progenitor cell sorting (using fluorescence-activated cell sorting and magnetic-activated cell sorting) and culture in tissue mimetic 3D matrix and niche-specific culture media, can yield tissue-specific organoids. Human ESCs and iPSCs can also be used to generate human organoids or create MPS. The applications of these systems are enormous and highly relevant to biomedical and clinical research. Using well-constructed organoid MPS can help understand inter-tissue/organ interaction under homeostasis and how these signaling gets dysregulated during disease progression

diseases. Ratiocinating from the prodigious prospects, it seems like s steady progressive demand for these advanced technologies is emerging, leading to a gradual and steady phase-out of animal-based experimentations.

Data availability No data is part of this paper.

Declarations

Conflict of interest The authors declare no conflict of interest.

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