

Landscape of human organoids: Ideal model in clinics and research

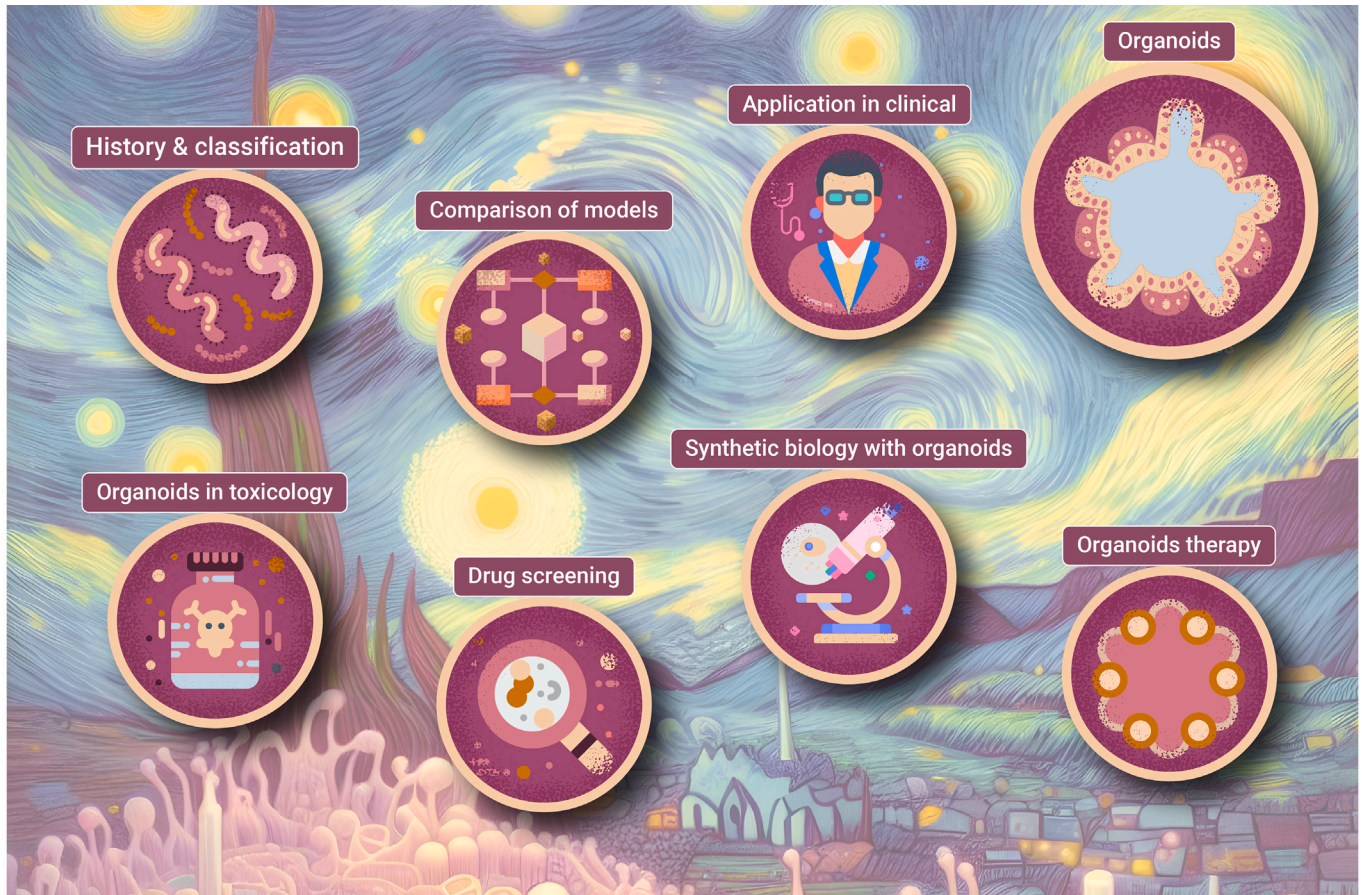
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- The landscape of organoids history: organoids mark a new and efficient model in tissue and organ level.
- Multiple applications of organoids in biomedicine and life healthcare.
- Organoids benefit to drug discovery, disease study, prevention, control, and therapy.
- Synthetic biology, artificial intelligence and automation integration broaden the role of organoids.



Landscape of human organoids: Ideal model in clinics and research

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In the last decade, organoid research has entered a golden era, signifying a pivotal shift in the biomedical landscape. The year 2023 marked a milestone with the publication of thousands of papers in this arena, reflecting exponential growth. However, amid this burgeoning expansion, a comprehensive and accurate overview of the field has been conspicuously absent. Our review is intended to bridge this gap, providing a panoramic view of the rapidly evolving organoid landscape. We meticulously analyze the organoid field from eight distinctive vantage points, harnessing our rich experience in academic research, industrial application, and clinical practice. We present a deep exploration of the advances in organoid technology, underpinned by our long-standing involvement in this arena. Our narrative traverses the historical genesis of organoids and their transformative impact across various biomedical sectors, including oncology, toxicology, and drug development. We delve into the synergy between organoids and avant-garde technologies such as synthetic biology and single-cell omics and discuss their pivotal role in tailoring personalized medicine, enhancing high-throughput drug screening, and constructing physiologically pertinent disease models. Our comprehensive analysis and reflective discourse provide a deep dive into the existing landscape and emerging trends in organoid technology. We spotlight technological innovations, methodological evolution, and the broadening spectrum of applications, emphasizing the revolutionary influence of organoids in personalized medicine, oncology, drug discovery, and other fields. Looking ahead, we cautiously anticipate future developments in the field of organoid research, especially its potential implications for personalized patient care, new avenues of drug discovery, and clinical research. We trust that our comprehensive review will be an asset for researchers, clinicians, and patients with keen interest in personalized medical strategies. We offer a broad view of the present and prospective capabilities of organoid technology, encompassing a wide range of current and future applications. In summary, in this review we attempt a comprehensive exploration of the organoid field. We offer reflections, summaries, and projections that might be useful for current researchers and clinicians, and we hope to contribute to shaping the evolving trajectory of this dynamic and rapidly advancing field.

INTRODUCTION

Organoid technology, the use of three-dimensional cultures derived from stem cells that closely mimic the architecture and functionality of native organs, represents a seminal advance in biomedical science, offering a revolutionary perspective on human physiology and pathology.¹ Organoid technology has profoundly impacted various fields, notably oncology and regenerative medicine, demonstrating unparalleled adaptability and precision.² Organoids, essentially miniature versions of organs, offer a highly physiologically relevant model for understanding human biology. This relevance is particularly critical in drug development, an area in which traditional models often fall short.³ Compared to two-dimensional cell cultures or animal models, organoids provide a more accurate representation of human tissues, enabling more reliable and efficient drug screening and functional validation.⁴ This feature is particularly valuable in the context of cancer research, where organoids can mimic the tumor micro-

environment, providing insights into tumor-immune interactions and host-pathogen dynamics.⁵ The clinical fidelity of organoids is higher than that of conventional models, as they can replicate the complex biological processes of human organs *in vitro*. This attribute enables the rapid functional testing of drugs, increasing the efficiency of the pathway from discovery to clinical application.⁶ Additionally, organoids present a novel platform for *in vitro* gene-editing therapies. By leveraging CRISPR-Cas9 and other gene-editing tools, researchers can use organoids to model genetic diseases and test therapeutic strategies, significantly advancing the field of personalized medicine.^{7,8} Our research findings emphasize the transformative potential of organoids in biomedical sciences. In the future, we expect organoids to play a pivotal role in advancing our understanding of human biology, evolving from cellular self-organization to integration with technologies such as synthetic biology and artificial intelligence (AI), offering substantial potential to revolutionize fields such as drug discovery, disease modeling, and personalized medicine. Progress in organoid technology continues to break barriers, offering insights once deemed unattainable. Accordingly, this review not only explores the current landscape of organoid technology but also offers a forward-looking perspective on the profound anticipated impact of organoids on future biomedical research.

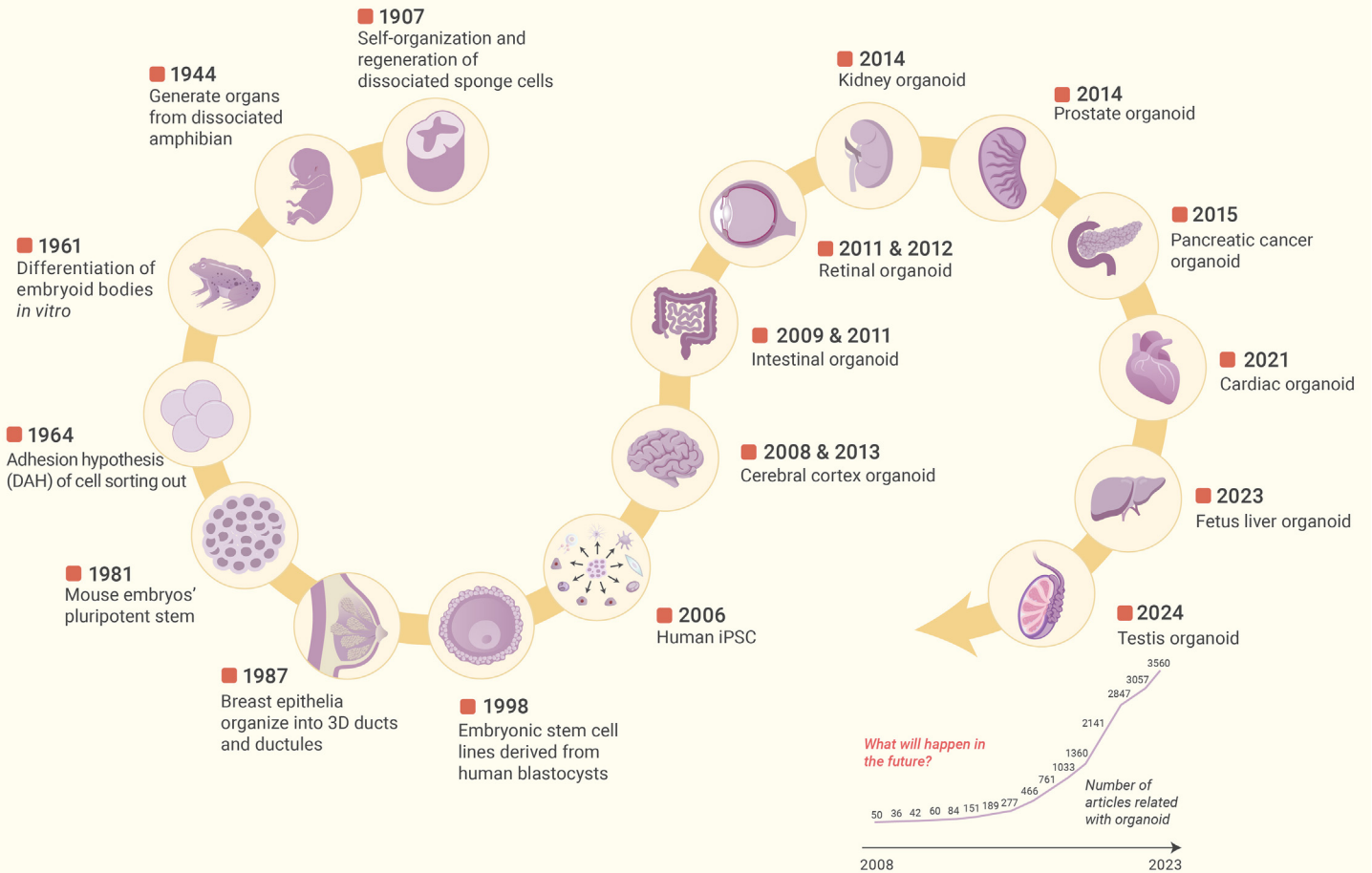
ORGANOID HISTORY

Organoid research, originating in the early 20th century, explores the self-organizing properties of cells (Figure 1A). The initial studies involved cultivating mechanically dissociated sponge cells to create functional organisms *in vitro*. H.V. Wilson's observations in 1907 marked the inception of organoid research, revealing the self-organization and regeneration of dissociated sponge cells.^{9–11} In subsequent decades, several groups performed dissociation-reaggregation experiments to generate different types of organs from dissociated amphibian pronephros and chick embryos,^{12–14} demonstrating the ability of cells to self-organize and form organ-like structures *in vitro*.

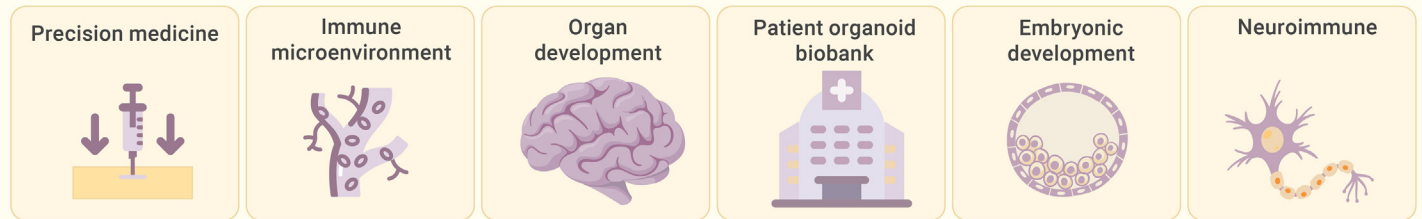
In 1981, pluripotent stem cells (PSCs) were isolated from mouse embryos, a milestone in stem cell research.^{15–18} In November 1998, human embryonic stem cells (ESCs) were derived from blastocysts, a breakthrough demonstrating human PSC derivation.¹⁹ The development of human induced pluripotent stem cell (iPSC) technology further revolutionized the field.^{20,21} The modern era of organoid technology truly began in the 2000s with the development of iPSCs by Shinya Yamanaka's team at Kyoto University^{22–25}; their pioneering work showed that mouse fibroblasts could be reprogrammed into a pluripotent state by introducing four transcription factors (Oct4, Sox2, Klf4, and c-Myc), generating the first iPSCs.^{18,22}

In 2009, Hans Clevers introduced the term "organoid," emphasizing self-organization, particularly in cancer research.²⁶ The term "organoid" draws inspiration from the ancient Greek lexicon, where "organon" is the origin of "organ" and the suffix "-oid" denotes resemblance or likeness. Therefore, "organoid" refers to something that resembles an organ. Researchers such as Lancaster and Knoblich expanded upon this seminal work.²⁷ They opined that the true essence of organoids lies not solely in their structural replication but in their ability to mirror the

A The development process of *in vitro* biological models: from cells to organoids



B Application of organoid model in medical and biology



C Source of organoids

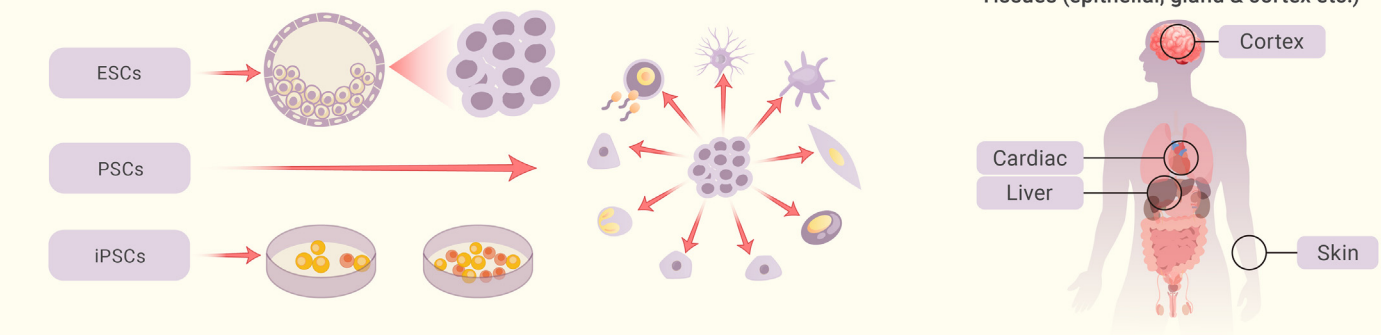


Figure 1. Unlocking organoids: A tale of historical discoveries and advanced taxonomies (A) The chronological history of the development of organoids. (B) Numerous novel applications of organoids have surfaced in the realm of life science research in recent years. (C) Further refinement is required in classification as a result of the diversification of organoid sources.

cellular diversity, specific functionalities, and even intricate cellular arrangements of the organs they represent.²⁸ Subsequently, the field of organoid technology advanced rapidly, ushering in a new era in scientific innovation.^{29,30} Hans Clevers' intestinal organoids paved the way for new research on intestinal biology and dis-

ease mechanisms³¹ and continue to be a valuable resource for investigating the molecular mechanisms underlying intestinal development and disease progression.^{26,32} Cerebral organoids, created by Lancaster et al. in 2013, mimic human brain development and aid in understanding developmental brain disorders.³³

In 2014, groundbreaking advances occurred in prostate organoid research, providing a robust platform for studying prostate cancer dynamics and therapeutic drug testing.^{34,35} In 2015, kidney organoids derived from iPSCs provided insights into kidney development and disease modeling.^{36,37} Subsequent years saw the burgeoning of organoid technology across various organ systems, including the pancreas. The development of pancreatic cancer organoids has illuminated the pathophysiological mechanisms that underlie pancreatic ductal adenocarcinoma, fostering advances in personalized medicine and targeted therapeutic strategies.^{38,39} A surge in methodological refinements in retinal organoid cultivation have occurred since 2018. These organoids recapitulate the functionality of retinal cells, representing a significant leap forward from earlier models that lacked structural and functional fidelity.^{40,41} Most recently, cardiac organoids emerged, aiding in modeling heart diseases.^{42,43}

Organoids have revolutionized biomedical research, enhancing drug assessments and personalized medicine.⁴⁴ In oncology, organoids have provided critical insights into the tumor immune microenvironment, propelling advancements in immunotherapy.⁴⁵ These three-dimensional cultures serve as pivotal tools in deciphering the complexities of organ and embryonic development, spotlighting essential elements of morphogenesis and the inception of life.⁴⁶ Moreover, the establishment of extensive organoid biobanks has supported personalized research approaches, offering a plethora of tissue-specific samples for in-depth study. In neuroimmune research, organoids have been instrumental in elucidating the intricate interplay between the nervous and immune systems, leading to new insights into a myriad of associated disorders.^{47,48} The remarkable ability of organoids to replicate organ-level functions within controlled environments is responsible for their indispensable role in precision medicine, disease modeling, and regenerative medicine, enabling a transformative phase in modern medical research (Figure 1B).

The field of organoid development utilizes a variety of stem cell sources, each with unique characteristics. Among these are the well-known ESCs and the revolutionary iPSCs, both of which fall under the broader category of PSCs (Figure 1C). The versatile mesenchymal stem cells also play a crucial role in this domain.^{49–55} Additionally, the world of clinical medicine offers a treasure trove of sources in the form of tissue specimens, both from individuals battling diseases and from healthy donors.^{56,57} This range includes surgical specimens and other less obvious sources such as nasopharyngeal swabs, which have recently gained prominence.⁵⁸

In conclusion, organoid research has introduced a new era in biomedical sciences, enhancing our understanding of life, disease, and therapies.

ORGANOIDS OUTSHINE CONVENTIONAL BIOMEDICAL MODELS

Biomedical researchers, in relentless pursuit of understanding life's complexity, have long sought models to mirror human physiology and pathology (Figure 2). Animal models, once pivotal in research,⁵⁹ present challenges due to interspecies differences and translational limitations.^{60–62} As the limitations of animal models grew more apparent, the use of cell lines became prevalent.⁶³ However, cell lines, though scalable, have the disadvantages of genetic homogeneity and two-dimensional constraints.^{64–66} In this complex scenario, the scientific community witnessed the emergence of organoids, three-dimensional structures derived from stem cells. These scaled-down models of organs are designed to bridge the gap between the simplicity of cell lines and the complexity of *in vivo* organisms, providing a clearer perspective on human biology.^{57,67}

Patient-derived organoids, which resemble the original tissues, hold promise in biomedical research. These models are established from patient samples, retain their genetic complexity, and offer a rapid and cost-effective way to study diseases.⁶⁸ Patient-derived organoids maintain interpatient heterogeneity, making them valuable for personalized medicine, especially in cancer research; however, their applications extend beyond oncology. For example, endometrial organoids have been successfully established from both normal and diseased human endometrium, including tissue samples representing conditions such as endometriosis, endometrial hyperplasia, and endometrial cancer.⁶⁹ These organoids maintain tissue characteristics and genetic stability, which can facilitate the understanding of disease heterogeneity. For instance, organoids from patients with Mayer-Rokitansky-Küster-Hausler syndrome have revealed unique gene-expression patterns, highlighting their potential for personalized medicine.⁷⁰ This emphasizes the potential of endometrial organoids to model individual patient pathologies and contribute to personalized medicine. Endometrial or-

ganoids have shown promise in high-throughput drug screening: they can be used to test the efficacy and toxicity of new therapeutic agents in a controlled environment that closely mimics *in vivo* conditions.⁶⁹ By testing drugs on patient-specific organoids, researchers can identify the most effective treatments for individual patients.⁶⁹ This personalized strategy has the potential to transform endometrial disease treatment by customizing therapies to each patient's specific needs, thereby advancing precision medicine.^{71,72}

In contrast, although patient-derived primary cell cultures closely resemble the original tumor,⁷³ they have a finite lifespan and limited replicative capacity. Their establishment can be challenging due to low success rates and slow growth, restricting the duration of drug-response testing.⁷⁴ Patient-derived xenograft models, which can take several months to establish and are costly to maintain, also exhibit genetic heterogeneity.⁷⁵ Moreover, the success rate of establishing these models can vary widely, making them less predictable than patient-derived organoids.⁷⁶ Genetically manipulated animal models are another alternative but present their own set of challenges.⁷⁷ These models take months or even years to develop, impose high maintenance costs, and exhibit genetic and cellular heterogeneity depending on the specific genetic modifications made.^{78–80} The rigidity and complexity of these models make them less adaptable than the more versatile and representative patient-derived organoids.

Organoid technology has advanced beyond other *in vitro* techniques to become a leading approach within the field of personalized medicine.⁸¹ Central to this advancement are iPSC-derived patient-specific organoids. These models have revolutionized our approach to understanding complex diseases by closely mirroring the genetic, cellular, and functional characteristics of a patient's tissue.^{82–85} iPSC-derived organoids offer unique advantages in disease modeling, particularly due to their patient-specific nature.⁸⁴ They allow personalized disease modeling, which is crucial in understanding complex diseases such as cancers, neurological disorders, and rare genetic diseases. Moreover, iPSCs are amenable to genetic editing using CRISPR-Cas9, enabling the study of disease mechanisms and the development of targeted therapies.^{82,83} iPSC-derived organoids have filled a gap in research opportunities by providing a more accurate, human-specific model, thereby revolutionizing the scope and methods of disease research. In Alzheimer's disease research, organoids replicate key disease features such as β -amyloid accumulation and tau protein buildup,^{86,87} offering insights into molecular mechanisms and genetic factors such as APOE4.⁸³ Similarly, Parkinson's disease research benefits from the use of midbrain-specific organoids in exploring interactions involving dopamine-producing neurons.^{88–91} The pathology of amyotrophic lateral sclerosis has been successfully mimicked in cerebral organoids using patient-derived protein extracts, illustrating TDP-43 aggregation and disease progression.⁹² This model has also demonstrated the time-dependent spread of pathogenic TDP-43, inducing astrogliosis, cellular apoptosis, and DNA double-strand breaks in the recipient cerebral organoids. Accordingly, this model enables the in-depth exploration of genetic mechanisms and the development of targeted gene therapies for amyotrophic lateral sclerosis.^{93–95}

The pharmaceutical industry, ever in search of therapeutic innovations, has embraced organoids as a powerful tool.^{96–98} Patient-specific organoids, with their personalized genetic tapestries, have proven to be robust platforms for drug testing, providing a path for individualized therapeutic blueprints. Their high accuracy, consistency, reproducibility, and fidelity in drug-screening paradigms support their potential as vanguards of drug discovery.^{99,100}

Furthermore, the convenience of subculturing and the potential for extended storage render organoids a favored option for extensive biobanking, accommodating a wide range of patient specimens.¹⁰¹ Because of their differences from and synergies with other models, organoids illuminate a path toward personalized medicine, drug discovery, and a more profound understanding of life itself.

ORGANOIDS SOLVE CLINICAL DIFFICULTIES IN ONCOLOGY

Organoid technology introduces a new generation of detailed and accurate simulations, capturing the genetic traits and heterogeneity inherent in complex diseases such as cancer.^{102–104} The ability of organoids to mimic diverse patient-specific responses is instrumental in dissecting the multifaceted nature of tumors, paving the way for targeted therapeutic interventions (Figure 3A). The detailed insights offered by these models are fostering new developments in biomedical research and pharmaceuticals, providing a novel approach to

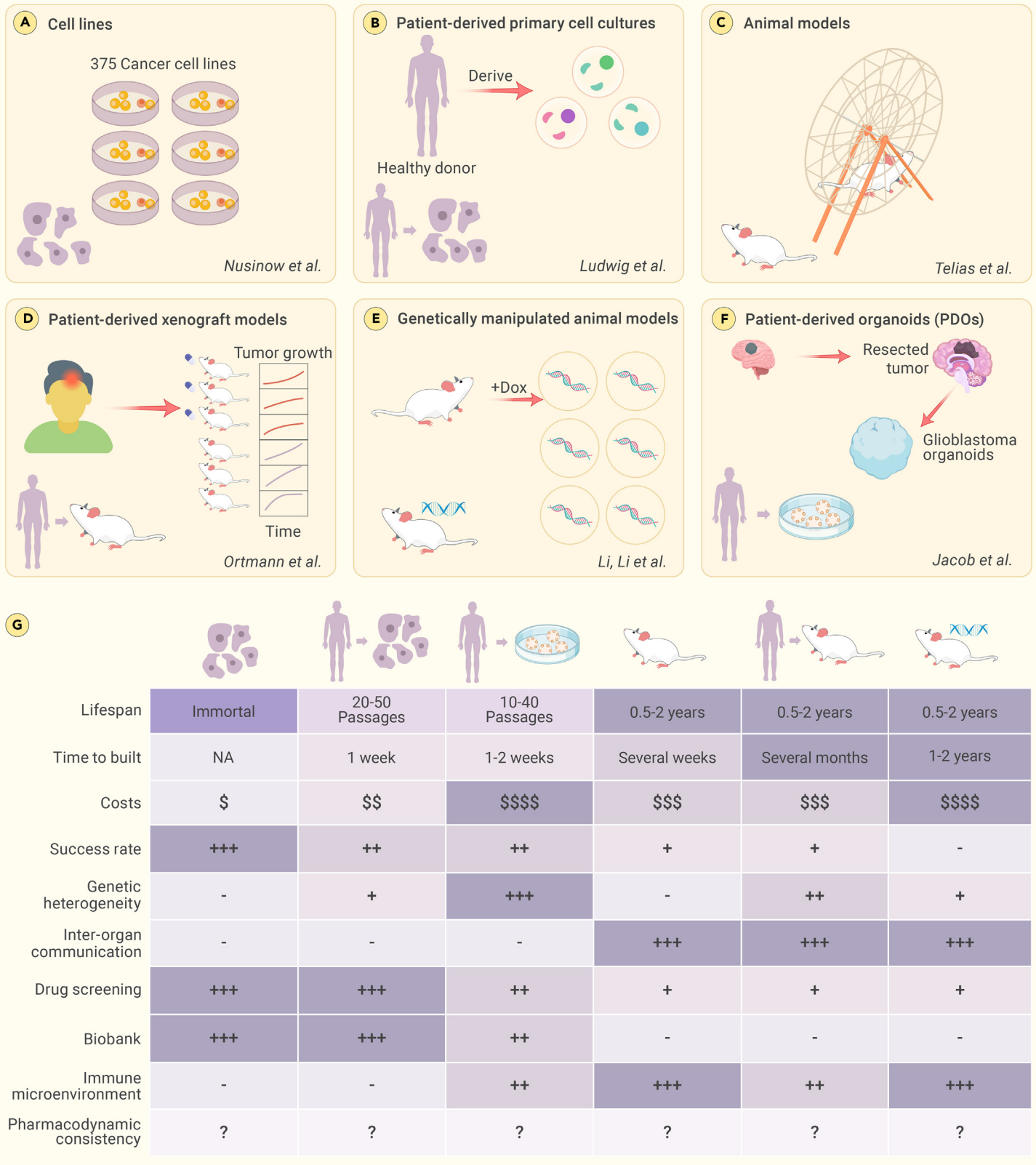


Figure 2. Organoids represent significant improvements over existing biomedical models Organoids, the miniaturized facsimiles of organs, have redefined the landscape of biomedical models, presenting a more physiologically relevant alternative. Compared to other models, organoids not only boast a shorter construction cycle and a higher success rate but also excel in preserving individualized tissue characteristics of patients.

address complex clinical challenges, such as pancreatic cancer,^{102,105} tumor drug resistance, recurrence, and metastasis.^{103,106,107}

Pancreatic cancer presents a significant challenge in oncology, as it is characterized by late diagnosis and limited treatment options, an aggressive nature, a dense stroma, high heterogeneity, and a lack of effective screening techniques.

These factors contribute to its poor prognosis as a clinically incurable disease.¹⁰⁸⁻¹¹⁰ The development of organoids, which are three-dimensional cell cultures replicating organ structure and function, introduces new possibilities in addressing this challenging cancer.¹⁰² Patient-derived organoids have been successfully derived from pancreatic ductal adenocarcinoma patients,

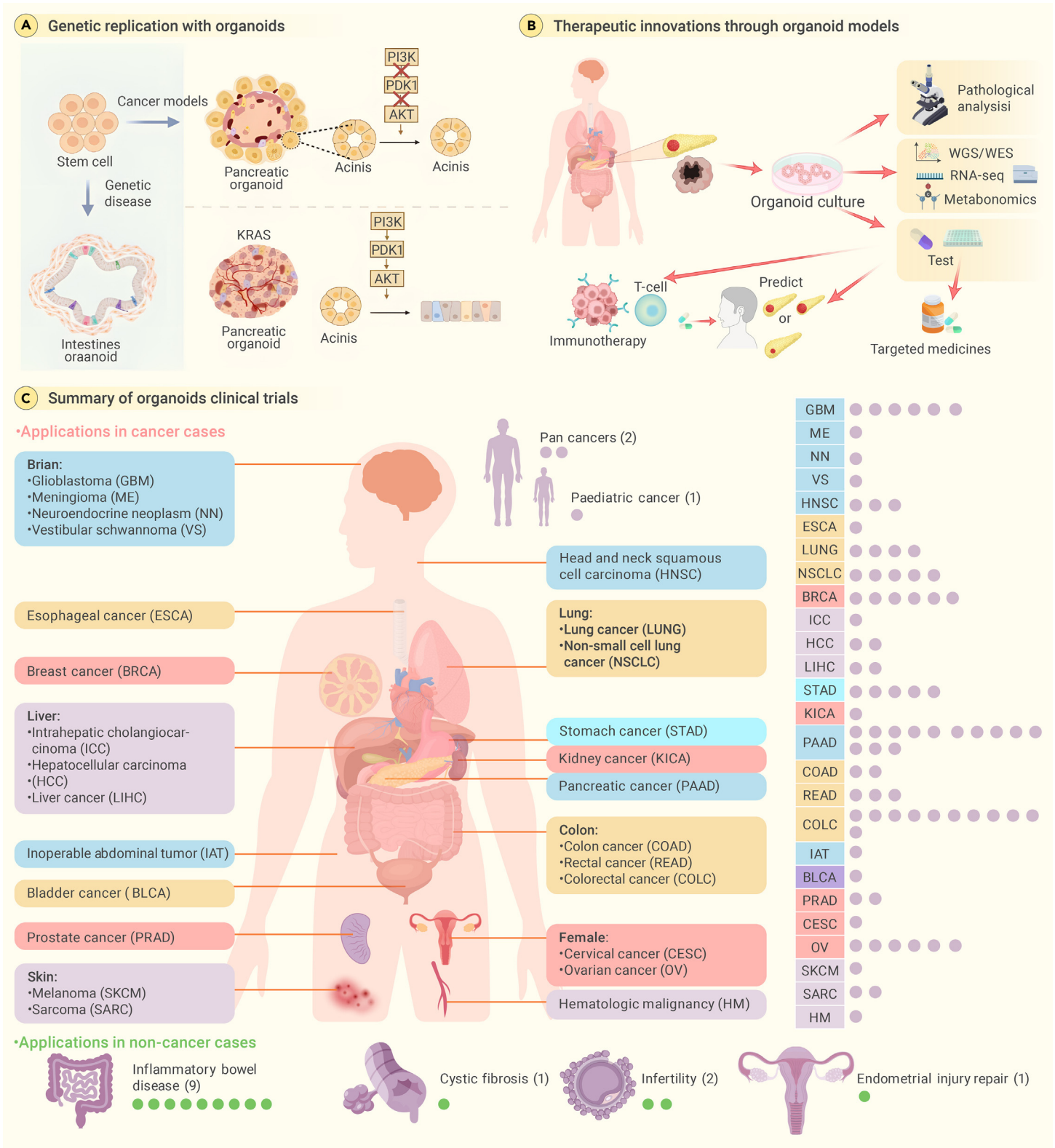


Figure 3. Organoid solves clinical difficulties in oncology: Pioneering models, therapies, and global trials (A) Utilizing organoids for genetic replication in a groundbreaking manner. Organoids are an innovative method that successfully generates genetically homogeneous models for cancers, specifically those that demonstrate resistance to medicine or repeated spread to other parts of the body. (B) Therapeutic innovations through organoid models. Utilizing the organoid-based genetic models, the scientific community is embarking on a journey of exploring and validating novel therapeutic regimens for tumors previously deemed untreatable. (C) Organoids at the forefront of global clinical endeavors. The global initiation of clinical trials that harness the capabilities of organoids testifies to their transformative potential.

demonstrating the ability to test the efficacy of various chemotherapy drugs.¹¹¹ In some cases, organoid drug responses have correlated with patient clinical responses.¹⁰² Promising results have been observed in drug-screening experiments, where kinase inhibitors, which are drugs that block certain enzymes called kinases, have been shown to decrease the viability of patient-derived orga-

noids, with CHK1 inhibitors exhibiting exceptional growth inhibition.¹⁰⁴ Organoids also show potential for treating drug-resistant tumors by maintaining key characteristics of primary tumors even after long-term passaging.^{103,112} They facilitate the exploration of resistance mechanisms related to cancer stem cells and can be used to study drug-resistance mechanisms, personalized medicine,

and high-throughput drug-screening methodologies. Organoids have been successfully established from multiple human tumor types, such as breast, pancreatic, gastrointestinal, lung, prostate, ovarian, and bladder cancers.¹⁰⁶ They can be used to target key genes and cancer stem cells to reverse drug resistance in cancer and have been shown to accurately predict patient responses to targeted therapies and chemotherapies (Figure 3B).¹¹³

Organoid models have emerged as a crucial asset in the field of immunotherapy, an innovative type of cancer therapy that leverages the body's immune system to identify and eradicate cancer cells.^{114–116} Organoid models have been instrumental in the generation and proliferation of tumor-reactive T cells derived from patient peripheral blood lymphocytes. Notably, these T cells exhibit a unique ability to target and annihilate autologous tumor organoids, emphasizing the promising role of organoids in the advancement of tailored immunotherapies.¹¹⁷ Moreover, organoids have proven crucial in forecasting individualized responses to immunotherapeutic interventions. Because they preserve the genetic integrity of the original tumor samples, organoids offer a highly representative model for assessing the effectiveness of various immunotherapeutic agents and their synergistic combinations.^{117,118} Research utilizing cancer organoids has shown that combination therapies markedly outperform monotherapy in terms of drug-response rates. This finding highlights the importance of organoids in refining treatment protocols and exploring novel therapeutic combinations.¹¹⁷ Furthermore, the cocultivation of organoids with immune cells, including tumor-infiltrating lymphocytes and peripheral blood mononuclear cells, has yielded valuable insights into the tumor microenvironment and its intricate interactions with the immune system. This methodology is vital for the evaluation of immunotherapies and for elucidating the dynamics of tumor-immune interplay.¹¹⁸ Advanced methodologies such as organoid-on-a-chip and three-dimensional bioprinting techniques have also been developed, enabling the creation of more elaborate cancer models.¹¹⁹ These sophisticated models enable precise manipulation of the tumor microenvironment and effectively simulate multiorgan metastases in cancer, providing a more accurate testing ground for immunotherapeutic agents.¹¹⁷ In summary, organoid models stand in the vanguard of cancer research and immunotherapy evaluation. This evolving technology is poised to become an essential instrument in the global battle against cancer, paving the way for more efficacious and personalized treatment for patients worldwide.^{120–122}

Organoid-based clinical trials are gaining momentum internationally, with significant contributions from countries including China and the United States (Figure 3C).¹ Clinical trials, prospective observations, and randomized controlled trials using organoids cover a wide range of diseases, including cystic fibrosis and various types of cancers such as breast cancer, colorectal cancer, lung cancer, esophageal cancer, and liver cancer. These trials are at the forefront of translating laboratory findings into clinical therapies to address some of the most daunting challenges in modern medicine.¹²³ Numerous applications of organoid technology are currently undergoing rigorous clinical evaluations and hold promise for a new era of personalized and effective treatment strategies.¹²⁴ These trials highlight the potential of organoids to provide tangible solutions to some of the most perplexing medical challenges of our time.¹²⁵ The integration of organoid technology into mainstream healthcare could revolutionize treatment modalities and present unprecedented opportunities to improve patient outcomes. As these clinical trials progress, they pave the way for the next generation of medical breakthroughs, powered by the sophisticated simulations that only organoid technology can provide.

THE INTERSECTION OF SYNTHETIC BIOLOGY AND ORGANOID TECHNOLOGY

The path to realizing the full potential of organoid technology is fraught with complexities and inconsistencies. These challenges, along with technical difficulties in creation and analysis, have somewhat impeded widespread adoption. Synthetic biology, however, is a field poised to surmount these hurdles. Synthetic biology is introducing a new era by connecting innovative methodologies to the multifaceted world of organoids.¹²⁶ This nexus between synthetic biology and organoid research is a rapidly growing field that is expected to overhaul our comprehension of biological systems and inaugurate unique therapeutic strategies.¹²⁷

Genetically encoded fluorescent biosensors are revolutionizing real-time monitoring in organoid development,¹²⁸ enabling the measurement of pro-

tein kinase activity, pH levels, and lactate levels.¹²⁹ From studying cancer formation and progression by monitoring specific biochemical markers to investigating neurological disorders through precise measurements, these tools offer powerful insights into disease at a molecular level.^{128,130} In gastrointestinal organoid culture, bioengineered sensors or biosensors have been integrated to enable measurements specific to gastrointestinal organoids.¹³¹

Advances in synthetic biology include the creation of tunable synthetic matrices using materials such as polyethylene glycol and hyaluronic acid to enhance organoid growth and functionality.¹³² Bioengineered hydrogels, such as those that emulate the esophageal microenvironment or support ovarian follicle culture, play crucial roles in promoting cell differentiation and organoid-like structure formation (Figure 4A). Synthetic biology, driven by advances in biomaterials such as hydrogels, is crucial for the development of organoid technology.¹³³ Cruz-Acuña et al. developed a bioengineered hydrogel system that mimics the esophageal microenvironment, promoting the differentiation of PSCs into esophageal epithelial cells.¹³⁴ Nason-Tomaszewski et al. created fibrous hydrogels to support organoid-like structure formation and oocyte survival in ovarian follicle culture.¹³⁵ Nguyen et al. utilized tryptophan zipper peptides to craft bioactive hydrogels with antimicrobial properties, promoting mammalian cell growth and inducing polarity changes in human intestinal organoids.¹³⁶ The concept of "assembloids" represents a further advance in bottom-up construction.¹³⁷ These organoids, derived from spatially organized multiple cell types, mimic the complexity of actual organs. By combining different cellular components, assembloid technology provides a high-throughput platform for generating functional human organoids. For example, researchers have created multilayer bladder assembloids that mimic the interactions between epithelium and stroma found in mature adult bladders.¹³⁸ These assembloids not only exhibit functional characteristics but also enable the modeling of specific conditions, such as urinary tract infections and urothelial carcinoma (Figure 4B).

Organoids also present a unique advantage in the field of synthetic biology, namely, the production of a myriad of biological products.¹³⁹ The unique milieu of these miniature organ structures mimics physiological conditions within the human body, facilitating the *ex vivo* production of complex molecules with high biocompatibility.¹⁴⁰ For example, organoid technology shows great promise in antibody production by mimicking immune system components to produce specific antibodies against pathogens *in vitro*. This accelerates therapeutic development and enables research into immune response.¹⁴¹ In another area of synthetic biology, organoid technology has revolutionized toxin production for medical research. The cultivation of organoids capable of producing snake venom is an important advance that offers potential for antivenom development, pharmacological research, and therapeutic applications. Organoids derived from snake venom gland cells provide an *in vivo*-like model for venom production.¹⁴² This study represents a significant stride in venom research, as these new organoids can serve as a viable platform that not only fosters a deeper understanding of venom biology but also propels the development of antivenoms and other therapeutic agents.¹⁴³ In summary, organoid technology is carving a new niche in the production of synthetic biology products and is expected to propel the development of innovative drugs and therapeutic strategies in the future (Figure 4C). Recently, Kim et al. reported another significant advance in the field of synthetic biology and organoid technology.^{144,145} Researchers created human enamel organoids from iPSCs to mimic tooth development. These organoids, which resemble ameloblasts, formed enamel in response to the presence of calcium and interacted with dental mesenchyme to enable mineralization. They also displayed differentiation potential and regenerative abilities. This advance in synthetic biology introduces new possibilities for dental treatments and research.^{144,145}

Synthetic biology, an evolving field, shows promise for the generation of diverse biochemical compounds, which is crucial for therapeutic development. The main challenge is efficiently identifying compounds with high biological activity and therapeutic value, and an emerging solution is using organoids in high-throughput screening systems. Organoids, which mimic the functions of real organs in three-dimensional structures, provide a platform for more accurately evaluating synthetic compounds. For instance, a study by Yang et al. emphasizes that organoids provide a three-dimensional

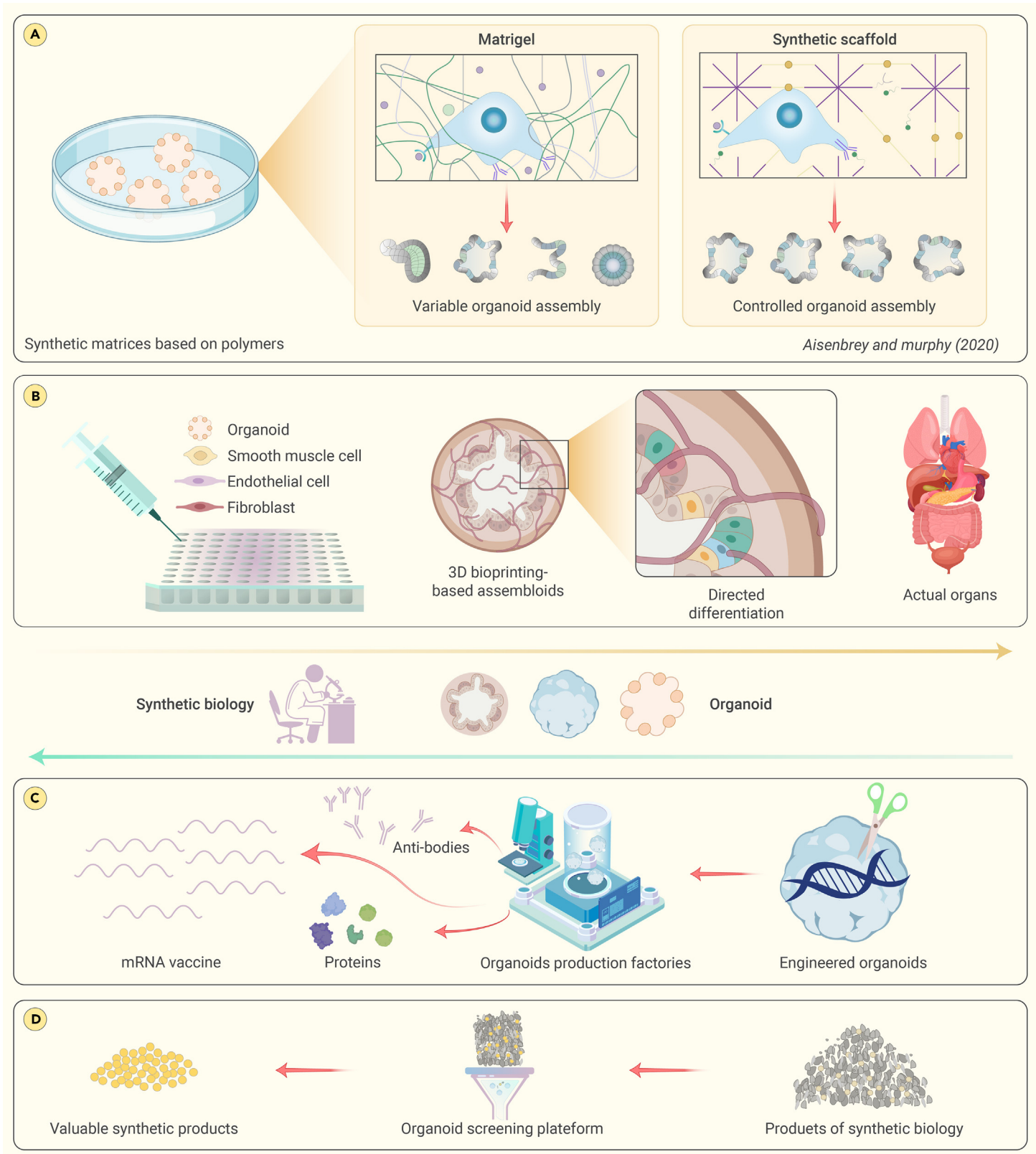


Figure 4. The intersection of synthetic biology and organoid (A) Synthetic biology aids in creating various auxiliary matrices and scaffolds. These structures provide an optimized environment, enhancing the growth and development of organoids. (B) By combining different cellular components, assembloids mimic the complexity of actual organs. (C) The utilization of organoid technology is establishing a distinct and specialized role within the realm of synthetic biology product manufacture. (D) Organoids, which are three-dimensional multicellular constructs that replicate the activity of actual organs, offer a distinct prospect for enhanced and streamlined screening procedures of synthetic drugs.

platform suitable for high-throughput drug screening, potentially bridging the gap between basic research and clinical practice.¹⁴⁶ The integration of compound production by synthetic biology and the screening capabilities of organoids could revolutionize therapeutic development. The synergy be-

tween generating diverse biological compounds and efficiently screening them for the most promising candidates could significantly expedite drug discovery, bringing effective treatments to patients more quickly and reliably (Figure 4D).

In conclusion, the fusion of synthetic biology and organoid research represents a monumental advance in biomedicine, presenting new avenues for studying and manipulating organoids. This intersection is poised to reshape our understanding of human biology and disease, paving the way for transformative clinical applications and developments in biomedical research.

ORGANOIDS: REVOLUTIONIZING THE LANDSCAPE OF TOXICOLOGY RESEARCH

Toxicology research, which is instrumental in evaluating the safety of pharmaceutical drugs, industrial chemicals, cosmetics, and food additives, has traditionally relied on animal models and two-dimensional cell cultures. The application of organoids in toxicology allows the exploration of tissue-specific responses to toxicants, which was hitherto impossible with the above traditional methods.¹⁴⁷ The primary use of organoid models in toxicology lies in drug development and toxicity testing.¹⁴⁸ Conventional *in vitro* and animal models frequently fail to precisely forecast toxicity in humans, causing late-stage drug failures and unforeseen adverse effects after market release. However, the ability to generate organoids that mimic the complexity and functionality of human organs presents new opportunities for understanding toxicity mechanisms at a cellular and molecular level. The significant contributions of organoids to toxicology include their use in drug-toxicity testing, where liver organoids have aided in predicting drug-induced liver injuries—a major cause of drug withdrawal from the market.¹⁴⁹

Liver organoids have proven to be a valuable *in vitro* tool for drug testing, including toxicity assessment.¹⁵⁰ Another study discusses the potential of using human PSC-derived organoids for high-fidelity drug-induced liver injury screening.¹⁵¹ Moreover, organoids derived from pluripotent or tissue-resident adult stem cells can self-organize into three-dimensional structures, closely mirroring the *in vivo* architecture and functionality of various organs.¹⁵² This ability allows organoids to mimic the effects of toxin damage on specific tissues, providing platforms for the specialized study of toxicity pathways. For instance, brain organoids comprising various neuronal and glial subtypes facilitate the analysis of the neurodevelopmental and neurodegenerative effects of drug toxicity.¹⁵³ Likewise, intestinal organoids have been utilized to study gastrointestinal toxicity, such as the mucosal damage induced by compounds like non-steroidal anti-inflammatory drugs.¹⁵⁴ In addition, the creation of patient-specific organoids exhibits tremendous potential in personalized medicine.¹⁴⁸ These organoids can help define individual susceptibility to various toxins, which could transform toxicity testing by facilitating personalized risk assessment and intervention strategies (Figure 5A).

The potential application of organoid models extends further to the field of environmental toxicology. Organoids facilitate research on how environmental toxins affect human health.¹⁴⁸ For instance, lung organoids have been used to study the impact of air-pollution particles on lung tissues, providing a realistic representation of human lung exposure to environmental hazards (Figure 5B).¹⁵⁵

Technological advances, including microfluidics and high-throughput screening systems, promise a bright future for organoids in toxicology. Microfluidic platforms can provide increased control over the organoid microenvironment,¹⁵⁶ fostering the examination of dynamic processes and cell interactions (Figure 5C). High-throughput screening systems can expedite the testing of numerous compounds, enabling efficient and cost-effective toxicological assessments. The incorporation of multiorganoid systems also shows great promise. By amalgamating different types of organoids, researchers can construct more intricate and interconnected models to simulate the interaction among organs in the human body.^{157,158} The system was able to demonstrate drug metabolism, interorgan crosstalk, and adverse drug reactions, offering a comprehensive assessment beyond what single-organ models can provide.¹⁵⁹ This strategy can enrich our understanding of systemic toxicity and the effects of toxins on multiple organs. Moreover, organoid engineering can help overcome some of the limitations of current organoid models. By incorporating additional cell types, such as immune or vascular cells, researchers can increase the physiological relevance of organoids and simulate the responses of human tissue to toxicants.¹⁴⁷ However, organoid models pose substantial challenges due to their complexity and variability. The differentiation of organoids into multiple cell types can fluctuate, leading to inconsistent study results.^{160,161} Additionally, the lack of vascularization in organoids can impede toxin delivery, skewing the interpretation of toxicological results.¹⁴⁷

In conclusion, organoid models in toxicology present immense potential. Addressing the challenges of standardization, scalability, physiological relevance, and ethical considerations will be pivotal to fully exploiting organoids in toxicology research. With continued technological advancement and interdisciplinary collaborations, organoid models present an opportunity to revolutionize toxicology, leading to more accurate and efficient testing of harmful substances and thereby significantly increasing human health and safety.

THE ORGANOID EPOCH: A PARADIGM SHIFT IN DRUG DEVELOPMENT

Organoid technology has played a pivotal role in the advancement and evaluation of new pharmaceuticals. While organoids are not directly involved in the creation of novel drugs, they are essential for facilitating the discovery of promising drug candidates via drug screening and testing.^{162,163} Specifically, organoids derived from colorectal cancer patients have been instrumental in assessing responses to inhibitors of tankyrases, enzymes that are crucial in Wnt signaling and are frequently disrupted in colorectal cancer. This testing has led to the identification of new drug candidates targeting the Wnt pathway.⁶⁸ Moreover, organoids from breast and lung cancer patients with certain mutations have been valuable in predicting sensitivity to PARP inhibitors, drugs that focus on DNA-repair mechanisms,^{68,164,165} contributing significantly to the development of individualized treatment plans involving PARP inhibitors. An additional study revealed that organoids possessing ARID1A missense mutations exhibited particular sensitivity to ATR inhibitors, which affect the DNA-damage response. This discovery holds significant promise for the development of new ATR-targeting drugs for cancers with ARID1A mutations.¹⁶⁶ Furthermore, organoids from endometrial and metastatic tumors responded to STAT3 inhibitors, hinting at the potential for novel drugs that target STAT3 signaling in endometrial adenocarcinoma.¹⁶⁷ Organoid lines from mesothelioma patients retained biomarkers and histological features of the patients' tumors.¹⁶⁸ In drug testing with cisplatin and pemetrexed, the organoids mirrored the patients' responses, demonstrating the potential of organoids in predicting treatment outcomes for mesothelioma. Finally, organoids from appendiceal carcinoma were used in immunotherapy trials, paving the way for the creation of new immunotherapeutic agents tailored to the unique characteristics of appendiceal tumors.¹⁶⁷ In every facet of pharmaceutical development, organoids manifest indispensable significance, providing robust support and verification from foundational research to clinical trials and all the way to the guidance of personalized medication, facilitating all-encompassing success in drug innovation.

The emergence of organoids in high-throughput drug screening has transformed drug discovery by enabling the targeted identification of effective molecules from large compound libraries.^{162,163} Recent advances in automation and systematic platforms have made organoids a gold standard in drug screening with the potential to streamline drug-discovery pipelines.^{169–171} Their ability to efficiently identify the most effective molecules from large compound libraries is transformative, and organoids are poised to become an integral part of drug-discovery pipelines. A large-scale organoid-based screening platform was used to test 1,172 Food and Drug Administration-approved compounds on pancreatic cancer organoids, and 22 drugs with anticancer effects were identified. This effort underscored the importance of three-dimensional culture-based platforms for drug screening, as some drugs that were effective in organoids did not show the same effects in two-dimensional cultures.¹⁶⁶ One of the most critical aspects of drug development is the early identification of toxicological profiles. Traditional animal models often fail to capture the full spectrum of human-specific toxicities, leading to costly failures in late-stage clinical trials. Organoids, because of their human-derived cells and tissue-specific architectures, constitute a more accurate model for assessing drug toxicity.^{172,173} Kidney organoids,^{174,175} for instance, have been used to evaluate nephrotoxicity, which is a significant concern in human pharmacology. Organoids are crucial and powerful tools not only for testing compound efficacy and toxicity but also for understanding disease mechanisms and validating therapeutic targets.¹⁷⁶ In cancer research, patient-derived tumor organoids play an important role in validating oncogenes and tumor-suppressor genes, thereby offering insights into drug mechanisms. In a study by Hirt et al.¹⁷⁷ reported by Mainardi and Bernards,¹⁶⁶ a newly derived pancreatic organoid biobank comprising 31 three-dimensional cell lines derived from tumors and nine from healthy tissue was used to investigate novel drug-gene interactions with potential translational value. The evaluation of drug responses in a context that mimics human physiology is crucial for the success

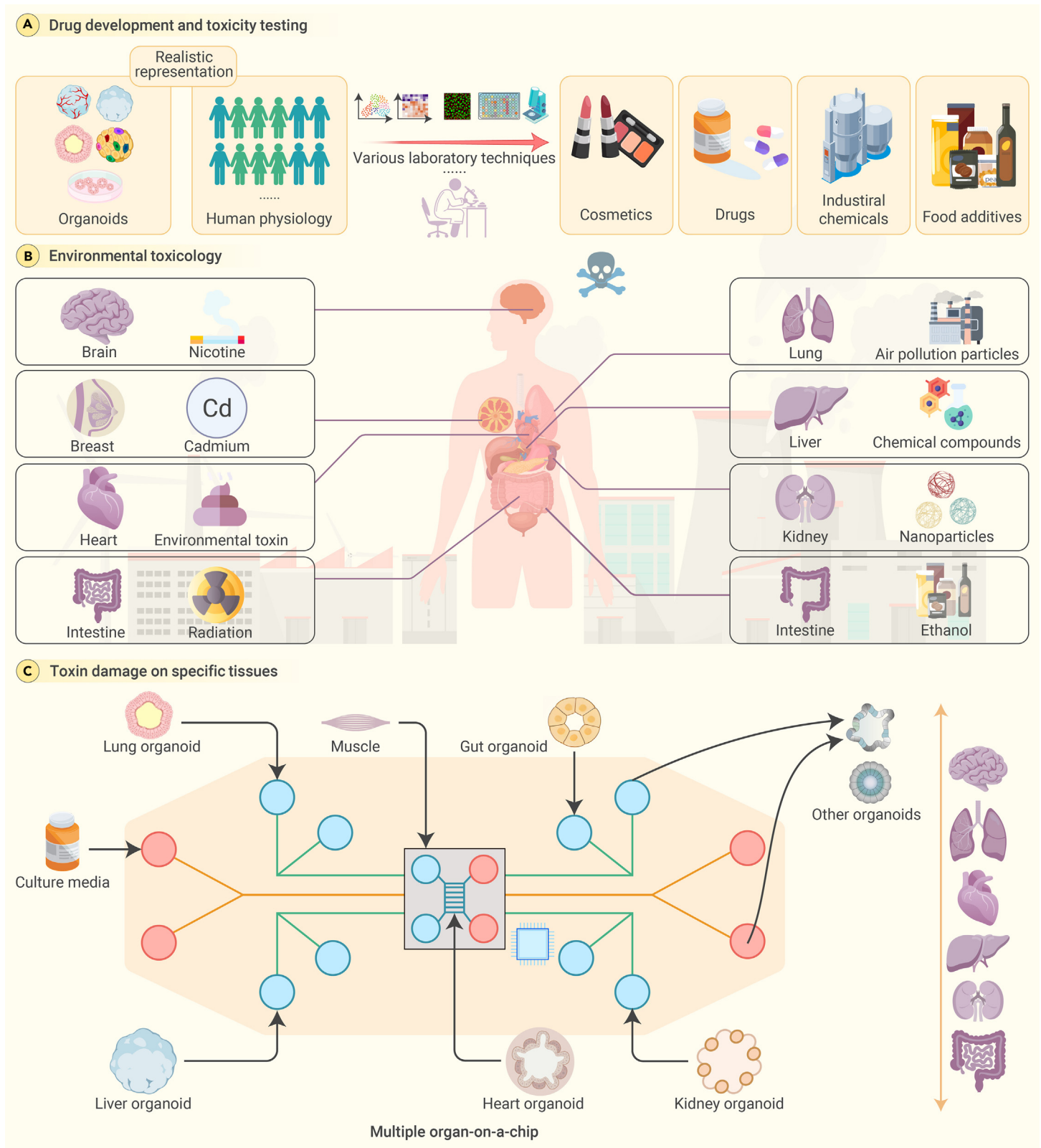


Figure 5. Organoids: Transforming the field of toxicology research (A) Organoids represent a significant advancement in accurately predicting human reactions to common items, surpassing the reliability of conventional models. Stem cell-derived organoids provide a more precise reproduction of human tissue structure and physiological reactions, making them suitable for assessing the safety of common consumer goods. (B) The environmental sentinel: organoids detecting global toxic threats. Organoids serve as powerful tools for detecting environmental toxins, including those affecting the brain. (C) Toward a comprehensive human model: multiorgan chips for effective toxicology. Microfluidic platforms and multiorganoid systems are at the forefront of simulating complex human physiology.

of any therapeutic intervention. The advantages of organoids in drug responses can even be applied to clinical trials of new drugs.^{81,178} Clinical trials are the bottleneck of drug development, with significant time and resource investments followed by high failure rates. Organoids have the potential to increase the suc-

cess rate of clinical trials by providing a more accurate representation of patients' diseased tissue and predicting their responses to specific therapies.¹⁷⁹ Organoids derived from patients enrolled in a trial could be used in parallel to evaluate drug responses, helping to adapt or modify the trial's course in real time. This

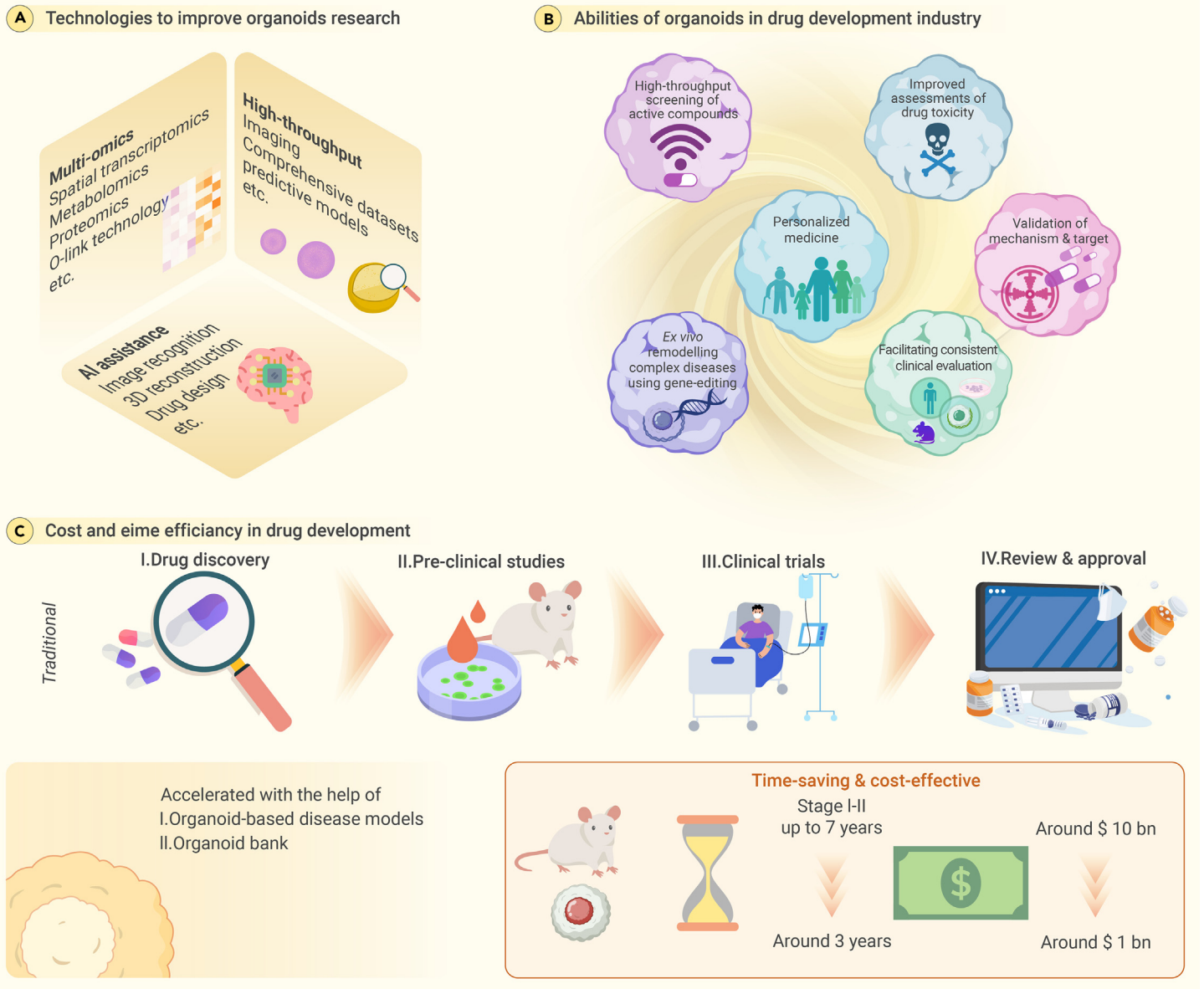


Figure 6. The organoid epoch: A paradigm shift in drug development (A) Organoid technology: advancing drug development across all stages. Organoids are crucial in influencing the drug-development sector, providing extensive assistance throughout the entire process. (B) The various applications of organoid models in biological research. Organoids play a pivotal role in the drug-development industry, offering a wide range of applications that streamline and enhance the process. (C) Comparing the cost and time efficiency of drug development: traditional methods with organoid-based approaches. Organoids represent a fundamental change in medication development, providing notable benefits compared to conventional approaches.

personalized approach to clinical trials holds great promise for improving patient outcomes and reducing the time and cost associated with drug development.¹⁸⁰ The aim of personalized medicine is to tailor treatments to individual patients based on their genetic and physiological characteristics. In oncology, patient-derived tumor organoids can be used to screen for effective chemotherapy agents, thereby personalizing treatment plans.^{180–183} The ability to evaluate drug responses in patient-specific organoids holds great promise for improving treatment outcomes and reducing adverse effects. Immunotherapies have revolutionized cancer treatment, but their efficacy varies among patients. Organoids constitute a powerful platform for studying the immune microenvironment and developing novel immunotherapies.^{184,185} Researchers can assess the efficacy of immunotherapeutic treatments by coculturing organoids with immune cells, such as tumor-infiltrating lymphocytes.¹⁸⁴ The study of tumor-infiltrating lymphocytes within organoids has also introduced new avenues for personalized immunotherapies tailored to the specific immunological landscape of each patient's tumor.^{186,187} The ability to model complex diseases *ex vivo* has been significantly advanced by the advent of organoid technology and gene-editing techniques. For instance, CRISPR-Cas9 has been instrumental in creating lung cancer models by introducing specific mutations into lung organoids.¹⁸⁸ Such

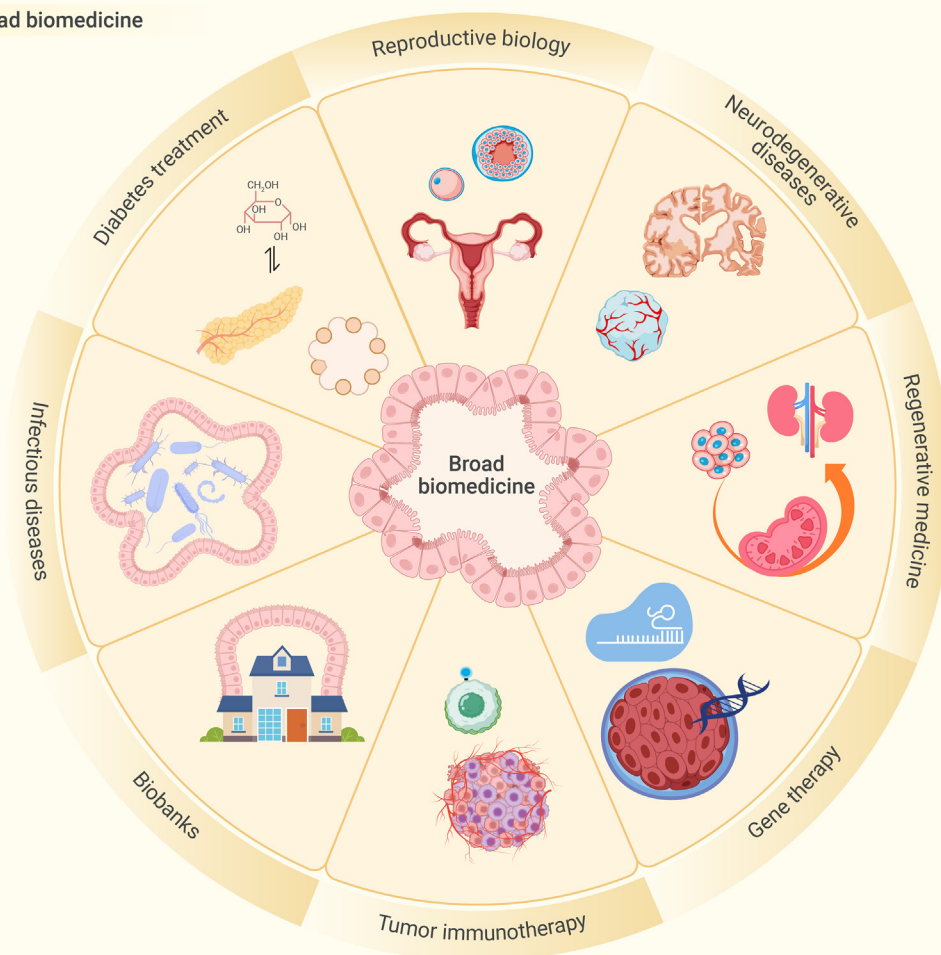
models offer a multifaceted approach to understanding complex diseases and have therefore been particularly useful in studying developmental and genetic diseases (Figures 6A and 6B).

Ex vivo modeling of complex diseases in organoids, aided by advanced gene-editing techniques, represents a cost-effective and ethical alternative to traditional drug-development methods. Organoids accurately replicate human physiology, thereby reducing the time and costs associated with drug discovery (Figure 6C).¹⁸⁹ Their utility in academic research and clinical application is significant, offering the potential to revolutionize the field and pave the way for more efficient therapeutic advancements.

ORGANOIDS ADVANCE BIOMEDICAL FIELDS ACROSS THE BOARD

The field of biomedical research has achieved a significant leap forward with the advent of organoid technology. Their versatility and adaptability make them a valuable tool for a wide range of research applications, with the potential to reshape therapeutic approaches to a range of clinical challenges (Figure 7A). The promise of organoids is particularly palpable in the realm of diabetes treatment. Researchers are abuzz regarding the creation of islet-like organoids that respond to glucose, which herald a potential paradigm shift in diabetes

A Organoids joint broad biomedicine



B Organoids expand the possibilities of disease models-practical cases

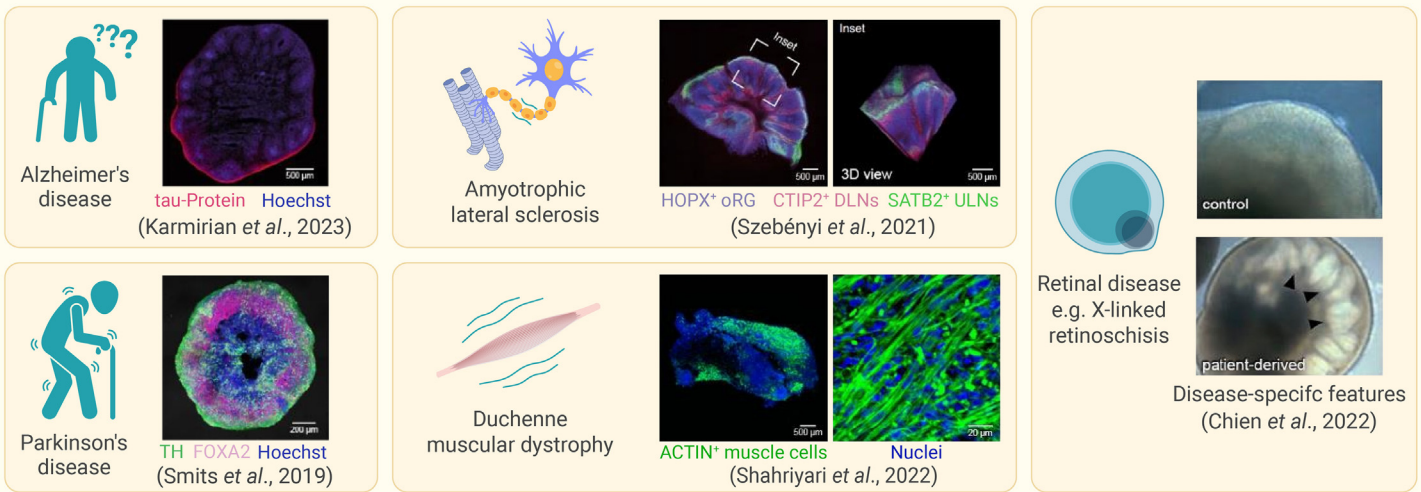


Figure 7. The multifaceted impact of organoid technology in biomedical science (A) The domain of biomedical research has experienced a substantial advancement with the introduction of organoid technology. The valuable attributes of versatility and adaptability possessed by these tools render them highly useful in a diverse array of research applications. Consequently, they hold great potential for revolutionizing therapeutic strategies aimed at addressing a wide range of clinical difficulties. (B) The application of organoids has greatly expanded the scope of disease inquiry. Technological advancements have made it possible to explore scientific realms that were previously inaccessible, especially when with regard to situations that are unique to humans.

management.¹⁹⁰ These organoids house a diverse array of cells: insulin-producing β cells, glucagon-producing α cells, somatostatin-producing δ cells, and pancreatic polypeptide-producing γ cells.¹⁹¹ Their efficacy was tested by transplantation into diabetic mice,¹⁹² where these organoids mimicked native pancreatic β cells, releasing insulin in response to blood glucose fluctuations and stabilizing glucose levels. Despite the promise of this approach, challenges remain,

such as scaling up organoid production and maintaining their long-term functionality.

Regenerative medicine, which is synonymous with restoration and healing, has found in organoids a robust and versatile ally.¹²⁵ These structures are pioneering advances in tissue repair,^{50,53,193,194} transplantation, and overall regenerative strategies.⁵³ In inflammatory bowel disease research, organoids are

providing insights into tissue-regeneration mechanisms, paving the way for innovative treatments.¹²⁵ Organoid transplantation is also presenting new possibilities. A prime example is the transplantation of human intestinal organoids into mice,¹⁹⁵ which resulted not only in the successful integration of the organoids but also in physiological improvements in the recipient mice. Similarly, research into human kidney organoids transplanted into rats has shown promising results,^{196,197} including enhanced vascularization and function. Such studies hint at a future in which organ transplant waiting lists could be significantly reduced, with organoids serving as a viable alternative. In biobanking, organoids are revolutionizing the collection, preservation, and utilization of normal and pathological specimens, improving disease modeling and drug screening.¹⁹⁸ Organoids also enable the preservation and study of viruses or microorganisms that are difficult to culture in traditional cell lines, providing more accurate models for research on tropism and immunity induction.^{199–201}

The intersection of organoids and infectious disease research is also particularly compelling. Organoids offer a dynamic platform for studying host-pathogen interactions, paving the way for deeper insights and innovative treatments.^{202–204} For instance, organoids have been pivotal in the study of respiratory viruses.²⁰⁵ Their application in research related to influenza and respiratory syncytial virus is just the tip of the iceberg.²⁰⁴ However, research on SARS-CoV-2 is where the potential of organoids is truly showcased.^{206–209} Researchers have used lung and intestinal organoids to gain a nuanced understanding of how the virus invades human cells.^{210–213} These studies are revealing vital data about viral entry, replication, and spread. Furthermore, organoids provide a platform for testing potential drugs against the virus, accelerating the drug-discovery process.^{214,215} Beyond viral infection, organoids have a role to play in bacterial and parasitic infection research.^{202,216} Their three-dimensional structure, mirroring that of human tissue, offers a unique environment to study the dynamics of bacterial infections at a cellular level. Additionally, in parasitic research, organoids are illuminating host-helminth interactions,²¹⁷ unveiling the intricate relationships between parasites and their human hosts.

Moreover, organoid models are a powerful tool for studying female fertility and reproductive biology. These organoids, derived from tissues in the female reproductive tract, enable research on normal reproductive processes and pathologies such as endometriosis, endometrial cancer, and ovarian cancer.^{156,218–220} Ovarian organoids facilitate the investigation of oocyte growth and maturation along with hormonal stimulation responses.²²⁰ Fallopian tube, endometrial, and cervical organoids are utilized to study various aspects of female reproductive health, including the development and function of the fallopian tubes, the menstrual cycle, endometriosis, endometrial hyperplasia, cervical clear cell carcinoma, and the impact of infections such as *Chlamydia trachomatis* and human papillomavirus. Additionally, organoids derived from pathological tissues have aided in understanding reproductive disorders and have been valuable for drug screening.^{156,218,220} Organoids also model early developmental events, such as implantation, and can be used to investigate interactions between different cell types such as trophoblast cells and the maternal decidua. Recent studies have also involved the development and characterization of trophoblast organoids and decidua organoids from human placental samples, which serve as models to study the maternal-fetal interface.²²¹ These organoids have been used to investigate innate immune signaling and the differential antiviral defenses present at the maternal-fetal interface. Moreover, organoids have been employed to study the effects of environmental toxicants on female fertility and reproductive health.²¹⁹ They offer a physiologically relevant and controlled system for the examination of intricate cellular processes within a contextually appropriate environment, offering the potential to revolutionize the field of reproductive medicine.

Duchenne muscular dystrophy, a debilitating genetic disorder, is also the subject of promising advances due to organoids. Using gene-editing technologies, researchers have been able to correct genetic mutations in iPSC-derived muscle cells, which constitutes a monumental advance in the development of effective treatments for Duchenne muscular dystrophy (Figure 7B).^{82,83} Organoid-based research has also enabled the development of innovative therapeutic strategies for retinal diseases, which have historically been challenging to treat.^{84,85} Leveraging organoids, scientists have been able to explore the potential of gene augmentation therapy using systems such as CRISPR-Cas9. One notable success has been the promise shown in treating Leber congenital amaurosis, a severe form of inherited blindness: targeted gene therapies have demonstrated potential in restoring vision.⁸³

In summary, the versatile applications of organoids are undeniably setting the stage for groundbreaking advancement in biomedical research. As we continue to harness their potential, the bridge between laboratory-based discoveries and real-world clinical applications is becoming more robust. The future promises a new era of personalized medicine with organoids at its core, offering hope for myriad clinical challenges.

TECH-INFUSED ORGANOID: THE NEW VANGUARD OF BIOMEDICINE

Recent technological advances are further empowering organoid systems and enabling the derivation of new biological insights. New tools in single-cell omics, imaging, genetics, and AI are being integrated with organoid platforms. Single-cell RNA sequencing (scRNA-seq) and other omics tools enable the high-resolution dissection of organoid composition and function (Figure 8).²²² Advanced imaging techniques allow the dynamic visualization of organoid morphogenesis and cell-cell interactions.²²³ AI and machine learning provide computational power to extract complex patterns and build predictive models from multidimensional organoid data.²²⁴ The integration of organoids with these emerging technologies has created a new generation of "organs-on-chips" that recapitulate tissue- and organ-level physiology in unprecedented detail.^{225,226}

Genetic engineering in organoids is a rapidly advancing field that combines organ-like complexity with molecular precision. Methods such as viral (retroviral, lentiviral, adenoviral) and nonviral (electroporation, lipofection) delivery systems enable gene manipulation in organoids,²²⁷ and CRISPR-Cas9 is revolutionizing precise gene editing.²²⁸ RNA interference is also used to modulate gene expression.²²⁷

Organoids are vital in high-throughput screening for assessing cell viability and metabolic activity. Enzyme-linked immunosorbent assays quantify biomarkers, while image-based profiling techniques such as epifluorescence and confocal microscopy are used to analyze organoid morphology and functional changes after treatment.^{163,229} High-content analysis dissects developmental processes and disease progression at the cellular level, integrating omics data and multidimensional imaging for a comprehensive view of organoid responses to drug treatments. z-stack imaging provides a complete cross-sectional view of three-dimensional organoids, increasing the accuracy of drug efficacy and toxicity assessments.²²⁹ Computational methods model cellular interactions and biomechanical properties in organoids, facilitating the optimization of culture conditions and the prediction of drug-treatment outcomes.²³⁰ Human liver organoids derived from PSCs have been successfully used to model drug-induced liver injury, aiding in antifibrosis drug screening and the identification of compounds with significant antifibrotic effects, such as SD208 and imatinib.²³⁰

The integration of single-cell omics and organoid technologies has significantly advanced our understanding of organ development, disease mechanisms, and potential therapeutic approaches. This synergy has been especially effective in the field of neurobiology and brain organoid research. Fleck et al. investigated the gene-regulatory network underlying human cerebral organoid development and used multiomics single-cell data to develop an algorithm called Pando to infer the gene-regulatory network, thereby revealing the involvement of different transcription factors at various stages of cerebral organoid development. Their research emphasizes the conservation of developmental programs across species and the predictive capability of multiregion human cerebral organoids as model systems.²³¹ Vento-Tormo emphasized the revolutionary upgrade that organoid technology,²³² combined with single-cell profiling, has brought to the field of human development. This combination allows a deeper understanding of cell-cell communication and cellular heterogeneity, as evidenced by the work of Camp et al. in studying human liver organoids using scRNA-seq.²³² Yin et al. further elaborated on the strengths of combining scRNA-seq and organoid technologies, highlighting applications such as the discovery of novel cell types and gene markers, the recapitulation of cellular heterogeneity, the delineation of cell-differentiation pathways, and the modeling of diseases.²³³ Single-cell omics technologies also enable the high-resolution dissection of cellular heterogeneity within organoid systems. scRNA-seq has been transformative in decoding organoid composition and identifying novel cell states. For example, scRNA-seq was used to map the cell lineages and transitional states in intestinal organoids, reconstructing the differentiation trajectories from stem cells to all intestinal epithelial cell types.²²² In patient-derived organoids, a recent study adopted an integrative approach combining scRNA-seq, epigenomic single-nucleotide polymorphism (SNP)-to-gene mapping, and genome-wide association study

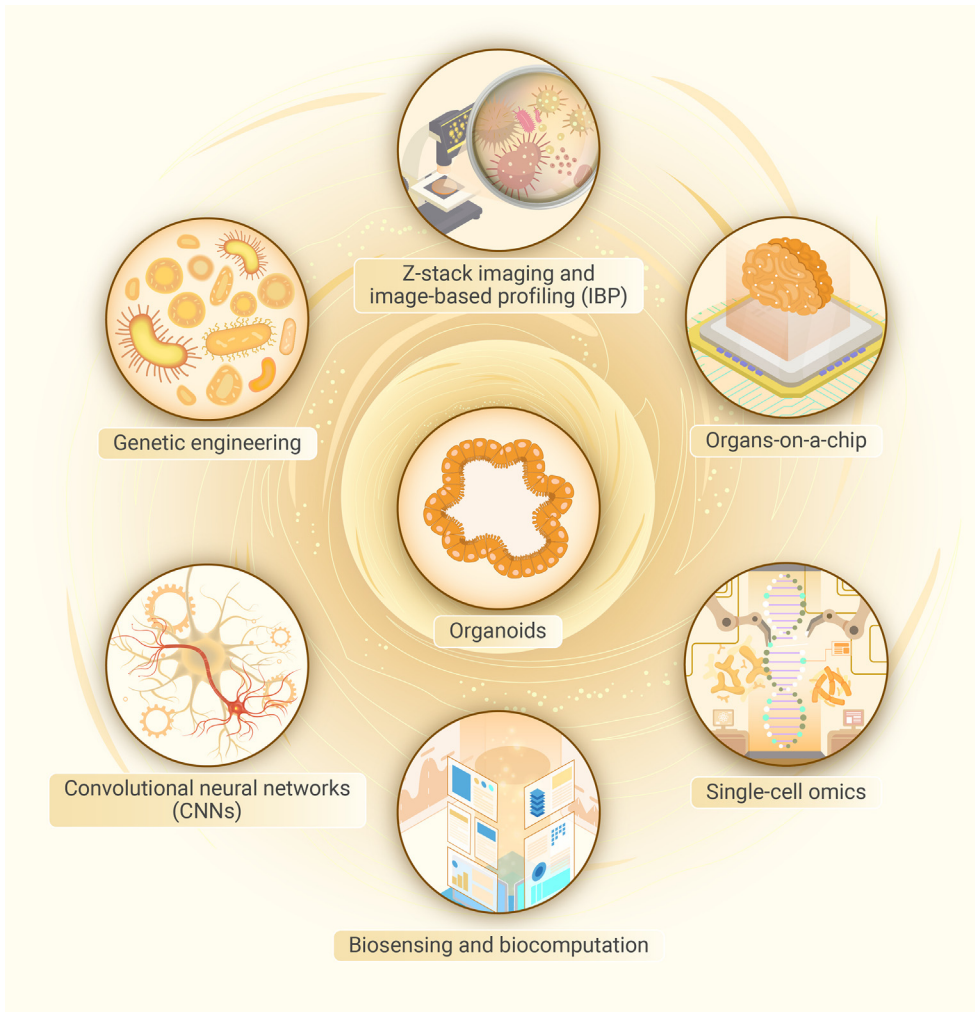


Figure 8. Tech-infused organoids: The cutting-edge advancement in biomedicine Organoids are now being enhanced with cutting-edge technical advancements. The integration of several disciplines in joint efforts has led to the development of organoids that are equipped with cutting-edge technology. This advancement holds the potential to bring about a significant transformation in the field of biomedicine.

Beyond merely visualizing images, AI can concurrently reconstruct multiple parameters from multi-omics data in organoids. These data are then integrated with complementary datasets, such as functional metrics and patient records, all derived from the same experimental conditions. The resulting repository is a large multimodal database. AI algorithms sift through this complex data to identify patterns and correlations, elucidating connections within diverse multiparametric information that may be challenging for human analysts to interpret. Overall, the integration of organoids with AI and deep learning constitutes an exciting new frontier.

The integration of organoids with microfluidics and sensors has enabled the development of “organs-on-chips” that recapitulate key aspects of human tissue physiology *in vitro*. These biomimetic systems incorporate organoids into micro-engineered devices with tissue-tissue interfaces, fluid flow, and real-time monitoring to model organ function and disease.²²⁵ One example is the gut-on-a-chip, which combines intestinal organoids with a microfluidic device lined by endothelial and immune cells to model the intestinal epithelium. This system faithfully reproduces the microenvironment of the living intestine,

including peristalsis-like motions and cyclic strain from breathing. Using this model, researchers showed that cyclic strain promotes intestinal stem cell differentiation and epithelial permeability. Thus, the gut-on-a-chip provides a powerful platform to study intestinal absorption, inflammation, gut-microbe interactions, and more.^{239–241} Another study by Campisi et al. presents a three-dimensional self-organized microvascular model of the blood-brain barrier using human iPSC-derived endothelial cells, brain pericytes, and astrocytes in a fibrin gel. This microfluidic system replicates *in vivo* neurovascular organization, exhibiting lower permeability than conventional *in vitro* models, and thus provides a physiologically relevant and robust platform for drug discovery and the prediction of neurotherapeutic transport efficacy.²⁴² Hajal et al. developed a human blood-brain barrier model within microfluidic devices by incorporating endothelial cells, brain pericytes, and astrocytes in a three-dimensional gel matrix. This model forms three-dimensional vessel architectures that resemble the natural blood-brain barrier, with gene-expression profiles and permeability values comparable to those observed *in vivo*.²⁴³ Accordingly, this blood-brain barrier model facilitates the quantitative assessment of molecular permeabilities and is suitable for use in both academic and industrial research to predict transport across the blood-brain barrier. These studies exemplify significant advances in organ-on-a-chip technology, specifically in modeling the blood-brain barrier.²⁴⁴ This progress underscores the potential of organ-on-a-chip technology to revolutionize biomedical research and therapeutic development.

Another exciting emerging application of organoids is the modeling of neural networks for biocomputation. Like their *in vivo* counterparts, the organized neuronal networks generated within brain organoids can perform specialized information processing.²⁴⁵ Researchers now plan to develop brain organoids that can serve as “biocomputers” for tasks such as pattern recognition and classification.²⁴⁶ In one pioneering study, a proof of concept of using brain organoids for biosensing and biocomputation was demonstrated. A recent publication in the journal *Neuron* revealed groundbreaking research conducted by Brett Kagan

summary statistics to deduce the cellular subtypes and molecular pathways by which genetic variants affect disease phenotypes.²³⁴ In summary, the integration of single-cell omics with organoid technologies provides a robust platform for modeling organ development and diseases. This combination has proven effective in revealing the cellular and molecular intricacies of organogenesis and disease pathogenesis.²³⁵ These advancements not only deepen our understanding of human biology but also pave the way for the development of novel therapeutic strategies.

The field of single-cell omics faces challenges in data interpretation due to the complexity of merging high-dimensional single-cell data with spatial information. The integration of AI and deep-learning algorithms can help streamline this process, enabling the identification of intricate patterns and interactions that are not easily discernible through traditional methods. Combining organoids with AI and deep learning enables innovative approaches to analyzing complex biological datasets and constructing predictive models. Organoids produce extensive multidimensional data, including cellular imaging, omics profiles, phenotypic readouts, and time series, and AI offers the computational power to extract meaningful patterns from this data overload.²³⁶

One major application is automated image analysis using convolutional neural networks, which can segment and classify cells in organoid imaging data orders of magnitude faster than human annotation.²³⁷ AI also enables phenotypic profiling from images. A deep-learning model was trained to predict the emergence of retinal tissue morphology and cell types in optic cup organoids over time.²³⁸ In the absence of reliable markers for assessing organoid vitality, researchers recently devised an innovative approach using AI algorithms to analyze a spectrum of phenotypic parameters. Remarkably, the AI-driven method can ascertain organoid aging solely through bright-field imaging. This approach eliminates the cumbersome steps of preparing single-cell suspensions and performing senescence-associated β -galactosidase staining, thereby streamlining quality control processes and making biobank operations more efficient.²²⁴

and his team at Cortical Labs, demonstrating that organoid neural networks cultured *in vitro* can learn to play the classic arcade game Pong.²⁴⁷ These so-called mini-brains, composed of interconnected neural cells, exhibit the ability to perceive and interact with their surrounding environment. Utilizing a computer interface, the neural networks were trained to detect the position of the game's digital sphere and manipulate a virtual paddle accordingly. Remarkably, the assemblage of brain cells acquired this skill within 5 min. Biocomputing with brain organoids could potentially be faster, more efficient, and more powerful than silicon-based computing and AI, requiring only a fraction of the energy.²⁴⁸ This technology could enable brain-inspired AI and powerful models for studying neural information processing.

Looking forward, the integration of organoids with new technologies has immense potential to transform biomedicine and human health. Such advances could power a new generation of preclinical disease models for drug screening, enable regenerative therapies using laboratory-grown tissues, and provide platforms for precision medicine. Realizing this potential will require collaborative multidisciplinary efforts at the intersection of stem cell biology, bioengineering, and data science. An exciting future lies ahead as organoids and emerging technologies combine to elucidate human biology in unprecedented detail.

CONCLUSION AND OUTLOOK

The advent of organoid technology marks a watershed in biomedical research, transcending previous boundaries of scientific inquiry. This innovation has revolutionized our understanding of human biology and introduced unprecedented avenues in disease management, addressing obstacles once considered insurmountable. At the cusp of this burgeoning field, the possibilities seem limitless, with organoid technology poised to reshape healthcare, research methodologies, and ethical considerations concerning human life.

Throughout this review, we have highlighted the multifaceted impact of organoids, emphasizing their utility in personalized medicine, drug discovery, and disease modeling. The bespoke nature of organoid systems, particularly patient-derived organoids, has introduced a new era of precision in biomedical research. By closely mimicking the intricate architecture and functionality of human organs, organoids offer a more representative model for human physiology and pathology, surpassing traditional *in vitro* and animal models. The capacity of organoids to replicate complex biological processes, such as the tumor immune microenvironment and host-pathogen interactions, positions them as a vanguard in further elucidating disease mechanisms and therapeutic responses.

Looking to the future, the integration of organoids with cutting-edge technologies such as synthetic biology, AI, and gene editing heralds a transformative phase in biomedical innovation. This synergy holds promise for unlocking new therapeutic pathways, advancing drug development, and providing profound new insights into complex diseases. The potential of organoids to serve as platforms for rapid drug testing and functional experimentation, coupled with their compatibility with gene-editing techniques, signifies a quantum leap in medical research.

In summary, organoid technology stands at the forefront of a revolution in the biomedical field. Its impact on human society will be profound, and its future applications appear boundless. As researchers and clinicians continue to harness the power of this technology, we anticipate a future replete with groundbreaking discoveries and innovative treatments that will ultimately reshape our approach to understanding and curing human diseases.

REFERENCES

- Yang, S., Hu, H., Kung, H., et al. (2023). Organoids: The current status and biomedical applications. *MedComm* **4**(3): e274. <https://doi.org/10.1002/mco2.274>.
- Barbuzano, J. (2017). Organoids: A new window into disease, development and discovery. *Harvard Stem Cell Institute News*. <https://hsci.harvard.edu/organoids>.
- Melzer, M.K., Resheq, Y., Navaee, F., et al. (2023). The application of pancreatic cancer organoids for novel drug discovery. *Expert Opin. Drug Discov.* **18**(4): 429–444. <https://doi.org/10.1080/17460441.2023.2194627>.
- Taverna, J.A., Hung, C.N., Williams, M., et al. (2024). Ex vivo drug testing of patient-derived lung organoids to predict treatment responses for personalized medicine. *Lung Cancer* **190**: 107533. <https://doi.org/10.1016/j.lungcan.2024.107533>.
- Tsai, S., McOlash, L., Palen, K., et al. (2018). Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models. *BMC Cancer* **18**(1): 335. <https://doi.org/10.1186/s12885-018-4238-4>.
- Kumar, S.S., Sreedharan, S.N., Patil, S., et al. (2020). The potential role of organoids in pathology and oncology research. *Pathol. Oncol. Res.* **26**(2): 1353–1354. <https://doi.org/10.1007/s12253-019-00642-z>.
- Driehuis, E., and Clevers, H. (2017). CRISPR/Cas 9 genome editing and its applications in organoids. *Am. J. Physiol. Gastrointest. Liver Physiol.* **312**(3): G257–G265. <https://doi.org/10.1152/ajpgi.00410.2016>.
- Duan, J., Penzes, P., and Gejman, P. (2019). From genetic association to disease biology: 2d and 3d human iPSC models of neuropsychiatric disorders and Crispr/Cas9 genome editing. *Eur. Neuropsychopharmacol.* **29**: S763–S764. <https://doi.org/10.1016/j.euroneuro.2017.06.120>.
- Eerkes-Medrano, D., Feehan, C.J., and Leys, S.P. (2015). Sponge cell aggregation: checkpoints in development indicate a high level of organismal complexity. *Invertebr. Biol.* **134**(1): 1–18. <https://doi.org/10.1111/ivb.12072>.
- Wilson, H.V. (1910). Development of sponges from dissociated tissue cells. *Bulletin of the Bureau of Fisheries* **30**: 1–35.
- Wilson, H.V. (1907). A new method by which sponges may be artificially reared. *Science* **25**(649): 912–915. <https://doi.org/10.1126/science.25.649.912>.
- Corrò, C., Novellasdemunt, L., and Li, V.S.W. (2020). A brief history of organoids. *Am. J. Physiol. Cell Physiol.* **319**(1): C151–C165. <https://doi.org/10.1152/ajpcell.00120.2020>.
- Okabayashi, K., and Asashima, M. (2006). In vitro organogenesis using amphibian pluripotent cells. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* **82**(7): 197–207.
- Zwilling, E. (1964). Development of fragmented and of dissociated limb bud mesoderm. *Dev. Biol.* **9**(1): 20–37. [https://doi.org/10.1016/0012-1606\(64\)90012-0](https://doi.org/10.1016/0012-1606(64)90012-0).
- Axelrod, H.R. (1984). Embryonic stem cell lines derived from blastocysts by a simplified technique. *Dev. Biol.* **101**(1): 225–228. [https://doi.org/10.1016/0012-1606\(84\)90133-7](https://doi.org/10.1016/0012-1606(84)90133-7).
- Evans, M.J., and Kaufman, M.H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292**(5819): 154–156. <https://doi.org/10.1038/292154a0>.
- Martin, G.R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci* **78**(12): 7634–7638. <https://doi.org/10.1073/pnas.78.12.7634>.
- Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**(4): 663–676. <https://doi.org/10.1016/j.cell.2006.07.024>.
- Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., et al. (1998). Embryonic stem cell lines derived from human blastocysts. *Science* **282**(5391): 1145–1147. <https://doi.org/10.1126/science.282.5391.1145>.
- Chang, E.A., Jin, S.W., Nam, M.H., et al. (2019). Human induced pluripotent stem cells: clinical significance and applications in neurologic diseases. *J. Korean Neurosurg. Soc.* **62**(5): 493–501. <https://doi.org/10.3340/jkns.2018.0222>.
- Chhabra, A. (2017). Derivation of human induced pluripotent stem cell (iPSC) lines and mechanism of pluripotency: historical perspective and recent advances. *Stem Cell Rev. Rep.* **13**(6): 757–773. <https://doi.org/10.1007/s12015-017-9766-9>.
- Takahashi, K., Tanabe, K., Ohnuki, M., et al. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**(5): 861–872. <https://doi.org/10.1016/j.cell.2007.11.019>.
- Ludwig, T.E., Kujak, A., Rauti, A., et al. (2018). 20 Years of human pluripotent stem cell research: it all started with five lines. *Cell Stem Cell* **23**(5): 644–648. <https://doi.org/10.1016/j.stem.2018.10.009>.
- Ochi, M. (2013). Shinya Yamanaka's 2012 Nobel Prize and the radical change in orthopedic strategy thanks to his discovery of iPSC cells. *Acta Orthop.* **84**(1): 1–3. <https://doi.org/10.3109/17453674.2013.765642>.
- Omole, A.E., and Fakoya, A.O.J. (2018). Ten years of progress and promise of induced pluripotent stem cells: historical origins, characteristics, mechanisms, limitations, and potential applications. *PeerJ* **6**: e4370. <https://doi.org/10.7717/peerj.4370>.
- Sato, T., Vries, R.G., Snippert, H.J., et al. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459**(7244): 262–265. <https://doi.org/10.1038/nature07935>.
- Lancaster, M.A., and Knoblich, J.A. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* **345**(6194): 1247125. <https://doi.org/10.1126/science.1247125>.
- Purnell, B.A. (2014). The making of bodies part by part. *Science* **345**(6194): 280. <https://doi.org/10.1126/science.345.6194.280-j>.
- Bender, E. (2015). Q&A: Hans Clevers. *Nature* **521**(7551): S15. <https://doi.org/10.1038/521S15a>.
- (2013). A gutsy approach to stem cells and signalling: an interview with Hans Clevers. *Dis Model Mech* **6**(5): 1053–1056. <https://doi.org/10.1242/dmm.013367>.
- Sinha, G. (2017). The organoid architect. *Science* **357**(6353): 746–749. <https://doi.org/10.1126/science.357.6353.746>.
- Pleguezuelos-Manzano, C., Puschhof, J., Van Den Brink, S., et al. (2020). Establishment and culture of human intestinal organoids derived from adult stem cells. *Curr. Protoc. Immunol.* **130**(1): e106. <https://doi.org/10.1002/cpim.106>.
- Lancaster, M.A., Renner, M., Martin, C.A., et al. (2013). Cerebral organoids model human brain development and microcephaly. *Nature* **501**(7467): 373–379. <https://doi.org/10.1038/nature12517>.
- (2014). Prostate cancer organoids make debut. *Cancer Discov.* **4**(11): 1248. <https://doi.org/10.1158/2159-8290.CD-NB2014-143>.
- Karthaus, W.R., Iaquineta, P.J., Drost, J., et al. (2014). Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* **159**(1): 163–175. <https://doi.org/10.1016/j.cell.2014.08.017>.
- Takasato, M., Er, P.X., Chiu, H.S., et al. (2015). Kidney organoids from human iPSCs contain multiple lineages and model human nephrogenesis. *Nature* **526**(7574): 564–568. <https://doi.org/10.1038/nature15695>.

37. Morizane, R., Lam, A.Q., Freedman, B.S., et al. (2015). Nephron organoids derived from human pluripotent stem cells model kidney development and injury. *Nat. Biotechnol.* **33**(11): 1193–1200. <https://doi.org/10.1038/nbt.3392>.
38. Boj, S.F., Hwang, C.I., Baker, L.A., et al. (2016). Model organoids provide new research opportunities for ductal pancreatic cancer. *Mol. Cell. Oncol.* **3**(1): e1014757. <https://doi.org/10.1080/23723556.2015.1014757>.
39. Huang, L., Holtzinger, A., Jagan, I., et al. (2015). Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. *Nat. Med.* **21**(11): 1364–1371. <https://doi.org/10.1038/nm.3973>.
40. Fligor, C.M., Langer, K.B., Sridhar, A., et al. (2018). Three-dimensional retinal organoids facilitate the investigation of retinal ganglion cell development, organization and neurite outgrowth from human pluripotent stem cells. *Sci. Rep.* **8**(1): 14520. <https://doi.org/10.1038/s41598-018-32871-8>.
41. Xian, B., Luo, Z., Li, K., et al. (2019). Dexamethasone provides effective immunosuppression for improved survival of retinal organoids after epiretinal transplantation. *Stem Cells Int.* **2019**: 7148032. <https://doi.org/10.1155/2019/7148032>.
42. Lewis-Israeli, Y.R., Wasserman, A.H., Gabalski, M.A., et al. (2021). Self-assembling human heart organoids for the modeling of cardiac development and congenital heart disease. *Nat. Commun.* **12**(1): 5142. <https://doi.org/10.1038/s41467-021-25329-5>.
43. Richards, D.J., Li, Y., Kerr, C.M., et al. (2020). Human cardiac organoids for the modelling of myocardial infarction and drug cardiotoxicity. *Nat. Biomed. Eng.* **4**(4): 446–462. <https://doi.org/10.1038/s41551-020-0539-4>.
44. Liu, L., Yu, L., Li, Z., et al. (2021). Patient-derived organoid (PDO) platforms to facilitate clinical decision making. *J. Transl. Med.* **19**(1): 40. <https://doi.org/10.1186/s12967-020-02677-2>.
45. Dominijanni, A., Mazzocchi, A., Shelkey, E., et al. (2020). Bioengineered tumor organoids. *Curr. Opin. Biomed. Eng.* **13**: 168–173. <https://doi.org/10.1016/j.cobme.2020.03.005>.
46. Hosseini, Z.F., Nelson, D.A., Moskwa, N., et al. (2019). Generating embryonic salivary gland organoids. *Curr. Protoc. Cell Biol.* **83**(1): e76. <https://doi.org/10.1002/cpcb.76>.
47. Sun, X.Y., Ju, X.C., Li, Y., et al. (2022). Generation of vascularized brain organoids to study neurovascular interactions. *Elife* **11**: e76707. <https://doi.org/10.1101/2022.01.04.474960>.
48. Fagerlund, I., Dougalis, A., Shakirzyanova, A., et al. (2021). Microglia-like cells promote neuronal functions in cerebral organoids. *Cells* **11**(1): 124. <https://doi.org/10.3390/cells11010124>.
49. Jensen, K.B., and Little, M.H. (2023). Organoids are not organs: sources of variation and misinformation in organoid biology. *Stem Cell Rep.* **18**(6): 1255–1270. <https://doi.org/10.1016/j.stemcr.2023.05.009>.
50. Turhan, A.G., Hwang, J.W., Chaker, D., et al. (2021). iPSC-derived organoids as therapeutic models in regenerative medicine and oncology. *Front. Med.* **8**: 728543. <https://doi.org/10.3389/fmed.2021.728543>.
51. McCauley, H.A., and Wells, J.M. (2017). Pluripotent stem cell-derived organoids: using principles of developmental biology to grow human tissues in a dish. *Development* **144**(6): 958–962. <https://doi.org/10.1242/dev.140731>.
52. Rowe, R.G., and Daley, G.Q. (2019). Induced pluripotent stem cells in disease modelling and drug discovery. *Nat. Rev. Genet.* **20**(7): 377–388. <https://doi.org/10.1038/s41576-019-0100-z>.
53. Kim, W., Gwon, Y., Park, S., et al. (2023). Therapeutic strategies of three-dimensional stem cell spheroids and organoids for tissue repair and regeneration. *Bioact. Mater.* **19**: 50–74. <https://doi.org/10.1016/j.bioactmat.2022.03.039>.
54. Lee, C.T., Bendriem, R.M., Wu, W.W., et al. (2017). 3D brain Organoids derived from pluripotent stem cells: promising experimental models for brain development and neurodegenerative disorders. *J. Biomed. Sci.* **24**(1): 59. <https://doi.org/10.1186/s12929-017-0362-8>.
55. Li, Z.A., Shang, J., Xiang, S., et al. (2022). Articular tissue-mimicking organoids derived from mesenchymal stem cells and induced pluripotent stem cells. *Organoids* **7**(2): 135–148. <https://doi.org/10.3390/organoids1020011>.
56. Azar, J., Bahmad, H.F., Daher, D., et al. (2021). The use of stem cell-derived organoids in disease modeling: an update. *Int. J. Mol. Sci.* **22**(14): 7667.
57. Kim, J., Koo, B.K., and Knoblich, J.A. (2020). Human organoids: model systems for human biology and medicine. *Nat. Rev. Mol. Cell Biol.* **21**(10): 571–584. <https://doi.org/10.1038/s41580-020-0259-3>.
58. Nio, Y., and Takebe, T. (2019). Organoid models of development and disease towards therapy. In *Medical Applications of iPSC Cells: Innovation in Medical Sciences*. Current Human Cell Research and Applications, H. Inoue and Y. Nakamura, eds. (Springer), pp. 149–168. https://doi.org/10.1007/978-981-13-3672-0_9.
59. Li, L., Bowling, S., McGeary, S.E., et al. (2023). A mouse model with high clonal barcode diversity for joint lineage, transcriptomic, and epigenomic profiling in single cells. *Cell* **186**(23): 5183–5199.e22. <https://doi.org/10.1016/j.cell.2023.09.019>.
60. Marshall, L.J., Bailey, J., Cassotta, M., et al. (2023). Poor translatability of biomedical research using animals – A narrative review. *Altern. Lab. Anim.* **51**(2): 102–135. <https://doi.org/10.1177/02611929231157756>.
61. Domínguez-Oliva, A., Hernández-Ávalos, I., Martínez-Burnes, J., et al. (2023). The importance of animal models in biomedical research: current insights and applications. *Animals* **13**(7): 1223. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10093480/>.
62. Barroca, N.C.B., Della Santa, G., Suchecki, D., et al. (2022). Challenges in the use of animal models and perspectives for a translational view of stress and psychopathologies. *Neurosci. Biobehav. Rev.* **140**: 104771. <https://doi.org/10.1016/j.neubiorev.2022.104771>.
63. Nusinow, D.P., Szpyt, J., Ghandi, M., et al. (2020). Quantitative proteomics of the cancer cell line encyclopedia. *Cell* **180**(2): 387–402.e16. <https://doi.org/10.1016/j.cell.2019.12.023>.
64. Lorsch, J.R., Collins, F.S., and Lippincott-Schwartz, J. (2014). Fixing problems with cell lines: technologies and policies can improve authentication. *Science* **346**(6216): 1452–1453. <https://doi.org/10.1126/science.1259110>.
65. Kaur, G., and Dufour, J.M. (2012). Cell lines: valuable tools or useless artifacts. *Spermatogenesis* **2**(1): 1–5. <https://doi.org/10.4161/spmg.19885>.
66. Li, J., Settivari, R.S., and LeBaron, M.J. (2019). Genetic instability of in vitro cell lines: implications for genetic toxicity testing. *Environ. Mol. Mutagen.* **60**(6): 559–562. <https://doi.org/10.1002/em.22280>.
67. Mead, B.E., and Karp, J.M. (2019). All models are wrong, but some organoids may be useful. *Genome Biol.* **20**(1): 66. <https://doi.org/10.1186/s13059-019-1677-4>.
68. Rae, C., Amato, F., and Braconi, C. (2021). Patient-derived organoids as a model for cancer drug discovery. *Int. J. Mol. Sci.* **22**(7): 3483. <https://doi.org/10.3390/ijms22073483>.
69. Gu, Z.Y., Jia, S.Z., Liu, S., et al. (2020). Endometrial organoids: A new model for the research of endometrial-related diseases. *Biol. Reprod.* **103**(5): 918–926. <https://doi.org/10.1093/biolre/iaaa124>.
70. Brucker, S.Y., Hentrich, T., Schulze-Hentrich, J.M., et al. (2022). Endometrial organoids derived from Mayer-Rokitansky-Küster-Hausler syndrome patients provide insights into disease-causing pathways. *Dis. Model. Mech.* **15**(5): dmm049379. <https://doi.org/10.1242/dmm.049379>.
71. Chen, P., Zhang, X., Ding, R., et al. (2021). Patient-derived organoids can guide personalized-therapies for patients with advanced breast cancer. *Adv. Sci.* **8**(22): 2101176. <https://doi.org/10.1002/advs.202101176>.
72. Boretto, M., Maenhoudt, N., Luo, X., et al. (2019). Patient-derived organoids from endometrial disease capture clinical heterogeneity and are amenable to drug screening. *Nat. Cell Biol.* **21**(8): 1041–1051. <https://doi.org/10.1038/s41556-019-0360-z>.
73. Ludwig, L.S., Lareau, C.A., Ulirsch, J.C., et al. (2019). Lineage tracing in humans enabled by mitochondrial mutations and single-cell genomics. *Cell* **176**(6): 1325–1339.e22. <https://doi.org/10.1016/j.cell.2019.01.022>.
74. Richter, M., Piwocka, O., Musielak, M., et al. (2021). From donor to the lab: a fascinating journey of primary cell lines. *Front. Cell Dev. Biol.* **9**: 711381. <https://doi.org/10.3389/fcell.2021.711381>.
75. Chen, X., Shen, C., Wei, Z., et al. (2021). Patient-derived non-small cell lung cancer xenograft mirrors complex tumor heterogeneity. *Cancer Biol. Med.* **18**(1): 184–198. <https://doi.org/10.20892/j.issn.2095-3941.2020.0012>.
76. Ortmann, J., Rampásek, L., Tai, E., et al. (2021). Assessing therapy response in patient-derived xenografts. *Sci. Transl. Med.* **13**(620): eabf4969. <https://doi.org/10.1126/scitranslmed.abf4969>.
77. Telias, M., Sit, K.K., Frozenfar, D., et al. (2022). Retinoic acid inhibitors mitigate vision loss in a mouse model of retinal degeneration. *Sci. Adv.* **8**(11): eabm4643. <https://doi.org/10.1126/sciadv.abm4643>.
78. Oh, B.Y., Hong, H.K., Lee, W.Y., et al. (2017). Animal models of colorectal cancer with liver metastasis. *Cancer Lett.* **387**: 114–120. <https://doi.org/10.1016/j.canlet.2016.01.048>.
79. Tagaya, H., Ishikawa, K., Hosokawa, Y., et al. (2019). A method of producing genetically manipulated mouse mammary gland. *Breast Cancer Res.* **21**(1): 1. <https://doi.org/10.1186/s13058-018-1086-8>.
80. Arends, M.J., White, E.S., and Whitelaw, C.B.A. (2016). Animal and cellular models of human disease. *J. Pathol.* **238**(2): 137–140. <https://doi.org/10.1002/path.4662>.
81. Takahashi, T. (2019). Organoids for drug discovery and personalized medicine. *Annu. Rev. Pharmacol. Toxicol.* **59**(1): 447–462. <https://doi.org/10.1146/annurev-pharmtox-010818-021108>.
82. Costamagna, G., Comi, G.P., and Corti, S. (2021). Advancing drug discovery for neurological disorders using iPSC-derived neural organoids. *Int. J. Mol. Sci.* **22**(5): 2659. <https://doi.org/10.3390/ijms22052659>.
83. Zhao, J., Fu, Y., Yamazaki, Y., et al. (2020). APOE4 exacerbates synapse loss and neurodegeneration in Alzheimer's disease patient iPSC-derived cerebral organoids. *Nat. Commun.* **11**(1): 5540. <https://doi.org/10.1038/s41467-020-19264-0>.
84. Olgasi, C., Cucci, A., and Follenzi, A. (2020). iPSC-derived liver organoids: A Journey from drug screening, to disease modeling, arriving to regenerative medicine. *Int. J. Mol. Sci.* **21**(17): 6215. <https://doi.org/10.3390/ijms21176215>.
85. Blaszkiewicz, J., and Duncan, S.A. (2022). Advancements in disease modeling and drug discovery using iPSC-derived hepatocyte-like cells. *Genes* **13**(4): 573. <https://doi.org/10.3390/genes13040573>.
86. Bubnys, A., and Tsai, L.H. (2022). Harnessing cerebral organoids for Alzheimer's disease research. *Curr. Opin. Neurobiol.* **72**: 120–130. <https://doi.org/10.1016/j.conb.2021.10.003>.
87. Karmirian, K., Holubiec, M., Goto-Silva, L., et al. (2023). Modeling Alzheimer's disease using human brain organoids. In *Alzheimer's Disease, 2561*, J. Chun, ed. (Springer US), pp. 135–158. https://doi.org/10.1007/978-1-0716-2655-9_7.
88. Galet, B., Cheval, H., and Ravassard, P. (2020). Patient-derived midbrain organoids to explore the molecular basis of Parkinson's disease. *Front. Neurol.* **11**: 1005. <https://doi.org/10.3389/fneur.2020.01005>.
89. McComish, S.F., MacMahon Copas, A.N., and Caldwell, M.A. (2022). Human brain-based models provide a powerful tool for the advancement of Parkinson's disease research and therapeutic development. *Front. Neurosci.* **16**: 851058. <https://doi.org/10.3389/fnins.2022.851058>.
90. Smits, L.M., Reinhardt, L., Reinhardt, P., et al. (2019). Modeling Parkinson's disease in midbrain-like organoids. *Npj Park Dis* **5**(1): 5. <https://doi.org/10.1038/s41531-019-0078-4>.
91. Smits, L.M., and Schwamborn, J.C. (2020). Midbrain organoids: a new tool to investigate Parkinson's disease. *Front. Cell Dev. Biol.* **8**: 359. <https://doi.org/10.3389/fcell.2020.00359>.
92. Tamaki, Y., Ross, J.P., Alipour, P., et al. (2023). Spinal cord extracts of amyotrophic lateral sclerosis spread TDP-43 pathology in cerebral organoids. *A.J. Myers, ed.* **19**(2): e1010606. <https://doi.org/10.1371/journal.pgen.1010606>.

93. Szebenyi, K., Wenger, L.M.D., Sun, Y., et al. (2021). Human ALS/FTD brain organoid slice cultures display distinct early astrocyte and targetable neuronal pathology. *Nat. Neurosci.* **24**(11): 1542–1554. <https://doi.org/10.1038/s41593-021-00923-4>.
94. Du, H., Huo, Z., Chen, Y., et al. (2023). Induced pluripotent stem cells and their applications in amyotrophic lateral sclerosis. *Cells* **12**(6): 971. <https://doi.org/10.3390/cells12060971>.
95. Ghasemi, M. (2022). Gene therapy in amyotrophic lateral sclerosis. *MDPI* **11**(13): 2066.
96. Miranda, C.C., and Cabral, J.M.S. (2020). Organoids for Cell Therapy and Drug Discovery. *Instituto Superior Técnico* **1**: 461–471. <https://linkinghub.elsevier.com/retrieve/pii/B9780128191781000459>.
97. Choo, N., Ramm, S., Luu, J., et al. (2021). High-throughput imaging assay for drug screening of 3D prostate cancer organoids. *SLAS Discov.* **26**(9): 1107–1124. <https://doi.org/10.1177/24725552211020668>.
98. Rauth, S., Karmakar, S., Batra, S.K., et al. (2021). Recent advances in organoid development and applications in disease modeling. *Biochim. Biophys. Acta. Rev. Cancer* **1875**(2): 188527. <https://doi.org/10.1016/j.bbcan.2021.188527>.
99. Ortolano, N. (2022). Organoids bring drug discovery and development to the culture hood. *10x Genomics*. <https://www.10xgenomics.com/blog/organoids-bring-drug-discovery-and-development-to-the-culture-hood>.
100. Urs, S. (2023). Organoid technology: a reliable tool for drug screening. *Crown Biosci Blog*. <https://blog.crownbio.com/organoid-technology-a-reliable-tool-for-drug-screening>.
101. Jacob, F., Salinas, R.D., Zhang, D.Y., et al. (2020). A patient-derived glioblastoma organoid model and biobank recapitulates inter- and intra-tumoral heterogeneity. *Cell* **180**(1): 188–204.e22. <https://doi.org/10.1016/j.cell.2019.11.036>.
102. Sereti, E., Papapostolou, I., and Dimas, K. (2023). Pancreatic cancer organoids: an emerging platform for precision medicine? *Biomedicine* **11**(3): 890. <https://doi.org/10.3390/biomedicine11030890>.
103. Pernik, M.N., Bird, C.E., Traylor, J.I., et al. (2021). Patient-derived cancer organoids for precision oncology treatment. *J. Pers. Med.* **11**(5): 423. <https://doi.org/10.3390/jpm11050423>.
104. Watanabe, S., Yogo, A., Otsubo, T., et al. (2022). Establishment of patient-derived organoids and a characterization-based drug discovery platform for treatment of pancreatic cancer. *BMC Cancer* **22**(1): 489. <https://doi.org/10.1186/s12885-022-09619-9>.
105. Choi, J.I., Jang, S.I., Hong, J., et al. (2021). Cancer-initiating cells in human pancreatic cancer organoids are maintained by interactions with endothelial cells. *Cancer Lett.* **498**: 42–53. <https://doi.org/10.1016/j.canlet.2020.10.012>.
106. Xu, H., Jiao, D., Liu, A., et al. (2022). Tumor organoids: applications in cancer modeling and potentials in precision medicine. *J. Hematol. Oncol.* **15**(1): 58. <https://doi.org/10.1186/s13045-022-01278-4>.
107. Nagle, P.W., Plukker, J.T.M., Muijs, C.T., et al. (2018). Patient-derived tumor organoids for prediction of cancer treatment response. *Semin. Cancer Biol.* **53**: 258–264. <https://doi.org/10.1016/j.semcancer.2018.06.005>.
108. Bazeed, A.Y., Day, C.M., and Garg, S. (2022). Pancreatic cancer: challenges and opportunities in locoregional therapies. *Cancers* **14**(17): 4257. <https://doi.org/10.3390/cancers14174257>.
109. Zeng, S., Pöttler, M., Lan, B., et al. (2019). Chemoresistance in Pancreatic Cancer. *Int. J. Mol. Sci.* **20**(18): 4504. <https://doi.org/10.3390/ijms20184504>.
110. Liot, S., Balas, J., Aubert, A., et al. (2021). Stroma involvement in pancreatic ductal adenocarcinoma: an overview focusing on extracellular matrix proteins. *Front. Immunol.* **12**: 612271. <https://doi.org/10.3389/fimmu.2021.612271>.
111. Piro, G., Agostini, A., Larghi, A., et al. (2021). Pancreatic cancer patient-derived organoid platforms: a clinical tool to study cell- and non-cell-autonomous mechanisms of treatment response. *Front. Med.* **8**: 793144. <https://doi.org/10.3389/fmed.2021.793144>.
112. Harada, K., and Sakamoto, N. (2022). Cancer organoid applications to investigate chemotherapy resistance. *Front. Mol. Biosci.* **9**: 1067207. <https://doi.org/10.3389/fmolb.2022.1067207>.
113. Su, C., Olsen, K.A., Bond, C.E., et al. (2023). The efficacy of using patient-derived organoids to predict treatment response in colorectal cancer. *Cancers* **15**(3): 805. <https://doi.org/10.3390/cancers15030805>.
114. Lockett, K.A., and Ganesh, K. (2023). Engineering the immune microenvironment into organoid models. *Annu. Rev. Cancer Biol.* **7**(1): 171–187. <https://doi.org/10.1146/annurev-cancerbio-061421-040659>.
115. Sun, C.P., Lan, H.R., Fang, X.L., et al. (2022). Organoid models for precision cancer immunotherapy. *Front. Immunol.* **13**: 770465. <https://doi.org/10.3389/fimmu.2022.770465>.
116. Wang, J., Chen, C., Wang, L., et al. (2022). Patient-derived tumor organoids: new progress and opportunities to facilitate precision cancer immunotherapy. *Front. Oncol.* **12**: 872531. <https://doi.org/10.3389/fonc.2022.872531>.
117. Fan, H., Demirci, U., and Chen, P. (2019). Emerging organoid models: leaping forward in cancer research. *J. Hematol. Oncol.* **12**(1): 142. <https://doi.org/10.1186/s13045-019-0832-4>.
118. Magré, L., Versteeg, M.M.A., Buschow, S., et al. (2023). Emerging organoid-immune co-culture models for cancer research: from oncimmunology to personalized immunotherapies. *J. Immunother. Cancer* **11**(5): e006290. <https://doi.org/10.1136/jitc-2022-006290>.
119. Saïding, Q., Ma, J., Ke, C., et al. (2022). From “organs on a chip” to “patient on a chip”. *Innovation* **3**(5): 100282. <https://doi.org/10.1016/j.xinn.2022.100282>.
120. Subtil, B., Iyer, K.K., Poel, D., et al. (2023). Dendritic cell phenotype and function in a 3D co-culture model of patient-derived metastatic colorectal cancer organoids. *Front. Immunol.* **14**: 1105244. <https://doi.org/10.3389/fimmu.2023.1105244>.
121. Zhu, Y., Kang, E., Wilson, M., et al. (2022). 3D tumor spheroid and organoid to model tumor microenvironment for cancer immunotherapy. *Organoids* **1**(2): 149–167. <https://doi.org/10.3390/organoids1020012>.
122. Miebach, L., Berner, J., and Bekeschus, S. (2022). In vivo model in cancer research and tumor immunology. *Front. Immunol.* **13**: 1006064. <https://doi.org/10.3389/fimmu.2022.1006064>.
123. Zhao, X., Jiang, Y., Liu, C., et al. (2022). Organoid technology and clinical applications in digestive system cancer. *Engineering* **9**: 123–130. <https://doi.org/10.1016/j.eng.2021.04.017>.
124. Takebe, T., Wells, J.M., Helmrich, M.A., et al. (2018). Organoid center strategies for accelerating clinical translation. *Cell Stem Cell* **22**(6): 806–809. <https://doi.org/10.1016/j.stem.2018.05.008>.
125. Choi, W.H., Bae, D.H., and Yoo, J. (2023). Current status and prospects of organoid-based regenerative medicine. *BMB Rep.* **56**(1): 10–14. <https://doi.org/10.5483/BMBRep.2022-0195>.
126. Glykofrydis, F., and Elfick, A. (2022). Exploring standards for multicellular mammalian synthetic biology. *Trends Biotechnol.* **40**(11): 1299–1312. <https://doi.org/10.1016/j.tibtech.2022.06.001>.
127. Sorrentino, G., Rezakhani, S., Yildiz, E., et al. (2020). Mechano-modulatory synthetic niches for liver organoid derivation. *Nat. Commun.* **11**(1): 3416. <https://doi.org/10.1038/s41467-020-17161-0>.
128. Ovechkina, V.S., Zakian, S.M., Medvedev, S.P., et al. (2021). Genetically encoded fluorescent biosensors for biomedical applications. *Biomedicine* **9**(11): 1528. <https://doi.org/10.3390/biomedicine9111528>.
129. Yousafzai, M.S., and Hammer, J.A. (2023). Using biosensors to study organoids, spheroids and organs-on-a-chip: a mechanobiology perspective. *Biosensors* **13**(10): 905. <https://doi.org/10.3390/bios13100905>.
130. Lin, W., Mehta, S., and Zhang, J. (2019). Genetically encoded fluorescent biosensors illuminate kinase signaling in cancer. *J. Biol. Chem.* **294**(40): 14814–14822. <https://doi.org/10.1074/jbc.REV119.006177>.
131. Kim, G.A., Ginga, N.J., and Takayama, S. (2018). Integration of sensors in gastrointestinal organoid culture for biological analysis. *Cell. Mol. Gastroenterol. Hepatol.* **6**(1): 123–131.e1. <https://doi.org/10.1016/j.jcmgh.2018.03.002>.
132. Poudel, H., Sanford, K., Szewo, P.K., et al. (2022). Synthetic matrices for intestinal organoid culture: implications for better performance. *ACS Omega* **7**(1): 38–47. <https://doi.org/10.1021/acsomega.1c05136>.
133. Zhang, Y., Li, D., Liu, Y., et al. (2024). 3D-bioprinted anisotropic bicellular living hydrogels boost osteochondral regeneration via reconstruction of cartilage–bone interface. *Innovation* **5**(1): 100542. <https://doi.org/10.1016/j.xinn.2023.100542>.
134. Cruz-Acuña, R., Kariuki, S.W., Sugijara, K., et al. (2023). Engineered hydrogel reveals contribution of matrix mechanics to esophageal adenocarcinoma and identifies matrix-activated therapeutic targets. *J. Clin. Invest.* **133**(23): e168146. <https://doi.org/10.1172/JCI168146>.
135. Nason-Tomaszewski, C.E., Thomas, E.E., Matera, D.L., et al. (2024). Extracellular matrix-templating fibrous hydrogels promote ovarian tissue remodeling and oocyte growth. *Bioact. Mater.* **3**: 292–303. <https://doi.org/10.1016/j.bioactmat.2023.10.001>.
136. Nguyen, A.K., Molley, T.G., Kardia, E., et al. (2023). Hierarchical assembly of tryptophan zipper peptides into stress-relaxing bioactive hydrogels. *Nat. Commun.* **14**(1): 6604. <https://doi.org/10.1038/s41467-023-41907-1>.
137. Huang, R., Shang, L., and Zhao, Y. (2021). Biomimic organ architectures and functions by assembling organoid models. *Sci. Bull.* **66**(9): 862–864. <https://doi.org/10.1016/j.scib.2021.02.008>.
138. Kim, E., Choi, S., Kang, B., et al. (2020). Creation of bladder assembloids mimicking tissue regeneration and cancer. *Nature* **588**(7839): 664–669. <https://doi.org/10.1038/s41586-020-3034-x>.
139. Mukherjee, A., Sinha, A., Maibam, M., et al. (2022). Organoids and commercialization. In *Organoid Bioengineering - Advances, Applications and Challenges*, 13, M.K. Paul, ed.. <https://doi.org/10.5772/intechopen.104706>.
140. Hernandez-Gordillo, V., Kassis, T., Lampejo, A., et al. (2020). Fully synthetic matrices for in vitro culture of primary human intestinal enteroids and endometrial organoids. *Biomaterials* **254**: 120125. <https://doi.org/10.1016/j.biomaterials.2020.120125>.
141. Wagar, L.E., Salahudeen, A., Constantz, C.M., et al. (2021). Modeling human adaptive immune responses with tonsil organoids. *Nat. Med.* **27**(1): 125–135. <https://doi.org/10.1038/s41591-020-11145-0>.
142. Sang, Y., Miller, L.C., Nelli, R.K., et al. (2021). Harness organoid models for virological studies in animals: a cross-species perspective. *Front. Microbiol.* **12**: 725074. <https://doi.org/10.3389/fmicb.2021.725074>.
143. Puschhof, J., Post, Y., Beumer, J., et al. (2021). Derivation of snake venom gland organoids for in vitro venom production. *Nat. Protoc.* **16**(3): 1494–1510. <https://doi.org/10.1038/s41596-020-00463-4>.
144. Kim, K.H., Kim, E.J., Kim, H.Y., et al. (2023). Fabrication of functional ameloblasts from hiPSCs for dental application. *Front. Cell Dev. Biol.* **11**: 1164811. <https://doi.org/10.3389/fcell.2023.1164811>.
145. Alghadeer, A., Hanson-Drury, S., Patni, A.P., et al. (2023). Single-cell census of human tooth development enables generation of human enamel. *Dev. Cell* **58**(20): 2163–2180.e9. <https://doi.org/10.1016/j.devcel.2023.07.013>.
146. Yang, J., Huang, S., Cheng, S., et al. (2021). Application of ovarian cancer organoids in precision medicine: key challenges and current opportunities. *Front. Cell Dev. Biol.* **9**: 701429. <https://doi.org/10.3389/fcell.2021.701429>.
147. Li, M., Gong, J., Gao, L., et al. (2022). Advanced human developmental toxicity and teratogenicity assessment using human organoid models. *Ecotoxicol. Environ. Saf.* **235**: 113429. <https://doi.org/10.1016/j.ecoenv.2022.113429>.
148. Caipa Garcia, A.L., Arlt, V.M., and Phillips, D.H. (2022). Organoids for toxicology and genetic toxicology: applications with drugs and prospects for environmental carcinogenesis. *Mutagenesis* **37**(2): 143–154. <https://doi.org/10.1093/mutage/geab023>.
149. Matsui, T., and Shinozawa, T. (2021). Human organoids for predictive toxicology research and drug development. *Front. Genet.* **12**: 767621. <https://doi.org/10.3389/FGENE.2021.767621>.

150. Haaker, M.W., Kruitwagen, H.S., Vaandrager, A.B., et al. (2020). Identification of potential drugs for treatment of hepatic lipidosis in cats using an in vitro feline liver organoid system. *J. Vet. Intern. Med.* **34**(1): 132–138. <https://doi.org/10.1111/jvim.15670>.
151. Shinozawa, T., Kimura, M., Cai, Y., et al. (2021). High-fidelity drug-induced liver injury screen using human pluripotent stem cell-derived organoids. *Gastroenterology* **160**(3): 831–846.e10. <https://doi.org/10.1053/j.gastro.2020.10.002>.
152. Truskey, G.A. (2018). Human microphysiological systems and organoids as in vitro models for toxicological studies. *Front. Public Health* **6**: 185. <https://doi.org/10.3389/fpubh.2018.00185>.
153. Seo, Y., Bang, S., Son, J., et al. (2022). Brain physiome: a concept bridging in vitro 3D brain models and in silico models for predicting drug toxicity in the brain. *Bioact. Mater.* **13**: 135–148. <https://doi.org/10.1016/j.bioactmat.2021.11.009>.
154. Shin, W., and Kim, H.J. (2022). 3D in vitro morphogenesis of human intestinal epithelium in a gut-on-a-chip or a hybrid chip with a cell culture insert. *Nat. Protoc.* **17**(3): 910–939. <https://doi.org/10.1038/s41596-021-00674-3>.
155. Winkler, A., Santo, N., Madaschi, L., et al. (2021). Lung organoids and microplastic fibers: a new exposure model for emerging contaminants. Preprint at bioRxiv. <https://www.biorxiv.org/content/10.1101/2021.03.07.432427v2>.
156. Heidari-Khoei, H., Esfandiari, F., Hajari, M.A., et al. (2020). Organoid technology in female reproductive biomedicine. *Reprod. Biol. Endocrinol.* **18**(1): 64. <https://doi.org/10.1186/s12958-020-00621-z>.
157. Lichou, F., and Trynka, G. (2020). Functional studies of GWAS variants are gaining momentum. *Nat. Commun.* **11**(1): 6283. <https://doi.org/10.1038/s41467-020-20188-y>.
158. Menon, R., and Richardson, L. (2022). Organ-on-a-chip for perinatal biology experiments. *Placenta Reprod. Med.* **1**: 98.
159. Ingber, D.E. (2022). Human organs-on-chips for disease modelling, drug development and personalized medicine. *Nat. Rev. Genet.* **23**(8): 467–491. <https://doi.org/10.1038/s41576-022-00466-9>.
160. Espinosa Angarica, V., and Del Sol, A. (2016). Modeling heterogeneity in the pluripotent state: a promising strategy for improving the efficiency and fidelity of stem cell differentiation. *Bioessays* **38**(8): 758–768. <https://doi.org/10.1002/bies.201600103>.
161. Hemeryck, L., Hermans, F., Chappell, J., et al. (2022). Organoids from human tooth showing epithelial stemness phenotype and differentiation potential. *Cell. Mol. Life Sci.* **79**(3): 153. <https://doi.org/10.1007/s00018-022-04183-8>.
162. Zhang, S., Shen, J., Wang, X., et al. (2023). Integration of organoids in peptide drug discovery: Rise of the high-throughput screening. *VIEW* **4**. <https://doi.org/10.1002/iviv.20230010>.
163. Lampart, F.L., Iber, D., and Doumpas, N. (2023). Organoids in high-throughput and high-content screenings. *Front. Chem. Eng.* **5**: 1120348. <https://doi.org/10.3389/fceng.2023.1120348>.
164. Campaner, E., Zannini, A., Santorsola, M., et al. (2020). Breast cancer organoids model patient-specific response to drug treatment. *Cancers* **12**(12): 3869. <https://doi.org/10.3390/CANCERS12123869>.
165. Kim, M., Mun, H., Sung, C.O., et al. (2019). Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening. *Nat. Commun.* **10**(1): 3991. <https://doi.org/10.1038/s41467-019-11867-6>.
166. Mainardi, S., and Bernards, R. (2022). A large-scale organoid-based screening platform to advance drug repurposing in pancreatic cancer. *Cell Genom.* **2**(2): 100100. <https://doi.org/10.1016/j.xgen.2022.100100>.
167. Yang, R., and Yu, Y. (2023). Patient-derived organoids in translational oncology and drug screening. *Cancer Lett.* **562**: 216180. <https://doi.org/10.1016/j.canlet.2023.216180>.
168. Ito, F., Kato, K., Yanatori, I., et al. (2023). Matrigel-based organoid culture of malignant mesothelioma reproduces cisplatin sensitivity through CTR1. *BMC Cancer* **23**(1): 487. <https://doi.org/10.1186/s12885-023-10966-4>.
169. Dhakal, B., Li, C.M.Y., Li, R., et al. (2022). The antiangiogenic drug perhexiline displays cytotoxicity against colorectal cancer cells in vitro: a potential for drug repurposing. *Cancers* **14**(4): 1043. <https://doi.org/10.3390/cancers14041043>.
170. Boehnke, K., Iversen, P.W., Schumacher, D., et al. (2016). Assay establishment and validation of a high-throughput screening platform for three-dimensional patient-derived colon cancer organoid cultures. *SLAS Discov* **21**(9): 931–941. <https://doi.org/10.1177/1087057116650965>.
171. Renner, H., Grabos, M., Becker, K.J., et al. (2020). A fully automated high-throughput workflow for 3D-based chemical screening in human midbrain organoids. *Elife* **9**: e52904. <https://doi.org/10.7554/eLife.52904>.
172. Karabici, M., Akbari, S., Ertem, O., et al. (2023). Human liver organoid models for assessment of drug toxicity at the preclinical stage. *Endocr., Metab. Immune Disord.: Drug Targets* **23**(14): 1713–1724. <https://doi.org/10.2174/1871530323666230411100121>.
173. Dilz, J., Auge, I., Groeneveld, K., et al. (2023). A proof-of-concept assay for quantitative and optical assessment of drug-induced toxicity in renal organoids. *Sci. Rep.* **13**(1): 6167. <https://doi.org/10.1038/s41598-023-33110-5>.
174. Karp, S., Pollak, M., and Subramanian, B. (2022). Disease modeling with kidney organoids. *Micromachines* **13**(9): 1384. <https://doi.org/10.3390/mi13091384>.
175. Astashkina, A.I., Mann, B.K., Prestwich, G.D., et al. (2012). Comparing predictive drug nephrotoxicity biomarkers in kidney 3-D primary organoid culture and immortalized cell lines. *Biomaterials* **33**(18): 4712–4721. <https://doi.org/10.1016/j.biomaterials.2012.03.001>.
176. Shamshirgaran, Y., Jonebring, A., Svensson, A., et al. (2021). Rapid target validation in a Cas9-inducible hiPSC derived kidney model. *Sci. Rep.* **11**(1): 16532. <https://doi.org/10.1038/s41598-021-95986-5>.
177. Hirt, C.K., Booi, T.H., Grob, L., Simmler, P., Toussaint, N.C., Keller, D., Taube, D., Ludwig, V., Goryachkin, A., and Pauli, C. (2022). Drug screening and genome editing in human pancreatic cancer organoids identifies drug-gene interactions and candidates for off-label treatment. *Cell Genom.* **2**: 100095. <https://doi.org/10.1016/j.xgen.2022.100095>.
178. Zhou, Z., Cong, L., and Cong, X. (2021). Patient-derived organoids in precision medicine: drug screening, organoid-on-a-chip and living organoid biobank. *Front. Oncol.* **11**: 762184. <https://doi.org/10.3389/fonc.2021.762184>.
179. Grossman, J.E., Muthuswamy, L., Huang, L., et al. (2022). Organoid sensitivity correlates with therapeutic response in patients with pancreatic cancer. *Clin. Cancer Res.* **28**(4): 708–718. <https://doi.org/10.1158/1078-0432.CCR-20-4116>.
180. Vlachogiannis, G., Hedayat, S., Vatsiou, A., et al. (2018). Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* **359**(6378): 920–926. <https://doi.org/10.1126/science.aao2774>.
181. Acklin-Wehnert, S., Dayanidhi, D., Czito, B.G., et al. (2023). Feasibility of establishing and drug screening patient-derived rectal organoid models from pretreatment rectal cancer biopsies. *J. Clin. Oncol.* **41**(4_suppl): 176. https://doi.org/10.1200/jco.2023.41.4_suppl.176.
182. Zhang, Y., Houchen, C.W., and Li, M. (2022). Patient-derived organoids pharmacotyping guides precision medicine for pancreatic cancer. *Clin. Cancer Res.* **28**(15): 3176–3178. <https://doi.org/10.1158/1078-0432.CCR-22-1083>.
183. Gerhards, N.M., and Rottenberg, S. (2018). New tools for old drugs: functional genetic screens to optimize current chemotherapy. *Drug Resist. Updat.* **36**: 30–46. <https://doi.org/10.1016/j.drup.2018.01.001>.
184. Neal, J.T., Li, X., Zhu, J., et al. (2018). Organoid modeling of the tumor immune microenvironment. *Cell* **175**(7): 1972–1988.e16. <https://doi.org/10.1016/j.cell.2018.11.021>.
185. Liu, C., Qin, T., Huang, Y., et al. (2020). Drug screening model meets cancer organoid technology. *Transl. Oncol.* **13**(11): 100840. <https://doi.org/10.1016/j.tranon.2020.100840>.
186. Zou, F., Tan, J., Liu, T., et al. (2021). The CD39 positive hepatitis B virus surface protein (HBVs)-targeted CAR-T and personalized tumor-reactive CD8+ T cells exhibit potent anti-hepatocellular carcinoma (HCC) activity. *Mol. Ther.* **29**(5): 1794–1807. <https://doi.org/10.1016/j.ymthe.2021.01.021>.
187. Sahin, U. (2018). Studying tumor-reactive T cells: a personalized organoid model. *Cell Stem Cell* **23**(3): 318–319. <https://doi.org/10.1016/j.stem.2018.08.015>.
188. Weidinger, P. (2017). Modeling Lung Tumorigenesis Using CRISPR/Cas9-Based Genome Editing in Ex Vivo 3D Organoids (University of Applied Sciences Technikum, Wien). PhD Thesis. https://www.marshallplan.at/images/All-Papers/MP-2017/Weidinger+Pia_747.PDF.
189. Weeber, F., Ooft, S.N., Dijkstra, K.K., et al. (2017). Tumor organoids as a pre-clinical cancer model for drug discovery. *Cell Chem. Biol.* **24**(9): 1092–1100. <https://doi.org/10.1016/J.CHEMBIOL.2017.06.012>.
190. Yoshihara, E., O'Connor, C., Gasser, E., et al. (2020). Immune-evasive human islet-like organoids ameliorate diabetes. *Nature* **586**(7830): 606–611. <https://doi.org/10.1038/s41586-020-2631-z>.
191. Tahbaz, M., and Yoshihara, E. (2021). Immune protection of stem cell-derived islet cell therapy for Treating Diabetes. *Front. Endocrinol.* **12**: 716625. <https://doi.org/10.3389/fendo.2021.716625>.
192. Zhou, J., and Huang, X. (2023). Scientists target human stomach cells for diabetes therapy. *WCM Newroom*. <https://news.weill.cornell.edu/news/2023/05/scientists-target-human-stomach-cells-for-diabetes-therapy>.
193. Arjmand, B., Rabbani, Z., Soveyzi, F., et al. (2023). Advancement of organoid technology in regenerative medicine. *Regen. Eng. Transl. Med.* **9**(1): 83–96. <https://doi.org/10.1007/s40883-022-00271-0>.
194. Qian, S., Mao, J., Liu, Z., et al. (2022). Stem cells for organoids. *Smart Med.* **1**(1): e20220007. <https://doi.org/10.1002/SMMD.20220007>.
195. Watanabe, S., Kobayashi, S., Ogasawara, N., et al. (2022). Transplantation of intestinal organoids into a mouse model of colitis - Nature Protocols. *Nat. Protoc.* **17**(3): 649–671. <https://doi.org/10.1038/s41596-021-00658-3>.
196. Grassi, L., Alfonsi, R., Francescangeli, F., et al. (2019). Organoids as a new model for improving regenerative medicine and cancer personalized therapy in renal diseases - Cell Death & Disease. *Cell Death Dis.* **10**: 201. <https://doi.org/10.1038/s41419-019-1453-0>.
197. Reviewed by Emily Henderson, B.S. (2022). World's first-in-human research using organoid transplantation yields good results. *News-Medicalnet*. <https://www.news-medical.net/news/20220822/Worlds-first-in-human-research-using-organoid-transplantation-yields-good-results.aspx>.
198. Boers, S.N., Van Delden, J.J., Clevers, H., et al. (2016). Organoid biobanking: identifying the ethics. *EMBO Rep.* **17**(7): 938–941. <https://doi.org/10.15252/embr.201642613>.
199. Sridhar, A., Simmini, S., Ribeiro, C.M.S., et al. (2020). A perspective on organoids for virology research. *Viruses* **12**(11): 1341. <https://doi.org/10.3390/v12111341>.
200. Lensink, M.A., Boers, S.N., Jongsma, K.R., et al. (2021). Organoids for personalized treatment of cystic fibrosis: professional perspectives on the ethics and governance of organoid biobanking. *J. Cyst. Fibros.* **20**(3): 443–451. <https://doi.org/10.1016/j.jcf.2020.11.015>.
201. Kim, M.B., Hwangbo, S., Jang, S., et al. (2022). Bioengineered co-culture of organoids to recapitulate host-microbe interactions. *Mater. Today. Bio* **16**: 100345. <https://doi.org/10.1016/j.mtbio.2022.100345>.
202. Roodsant, T., Navis, M., Aknouch, I., et al. (2020). A human 2D primary organoid-derived epithelial monolayer model to study host-pathogen interaction in the small intestine. *Front. Cell. Infect. Microbiol.* **10**: 272. <https://doi.org/10.3389/fcimb.2020.00272>.
203. Pellegrino, E., and Gutierrez, M.G. (2021). Human stem cell-based models for studying host-pathogen interactions. *Cell Microbiol.* **23**(7): e13335. <https://doi.org/10.1111/cmi.13335>.
204. Dutta, D., and Clevers, H. (2017). Organoid culture systems to study host–pathogen interactions. *Curr. Opin. Immunol.* **48**: 15–22. <https://doi.org/10.1016/j.coi.2017.07.012>.
205. Hong, K.J., and Seo, S.H. (2018). Organoid as a culture system for viral vaccine strains. *Clin. Exp. Vaccine Res.* **7**(2): 145–148. <https://doi.org/10.7774/cevr.2018.7.2.145>.

206. Yang, J., Gong, Y., Zhang, C., et al. (2022). Co-existence and co-infection of influenza A viruses and coronaviruses: public health challenges. *Innovation* **3**(5): 100306. <https://doi.org/10.1016/j.xinn.2022.100306>.
207. Li, X.F., Cui, Z., Fan, H., et al. (2022). A highly immunogenic live-attenuated vaccine candidate prevents SARS-CoV-2 infection and transmission in hamsters. *Innovation* **3**(2): 100221. <https://doi.org/10.1016/j.xinn.2022.100221>.
208. Liu, W., Jia, J., Dai, Y., et al. (2022). Delineating COVID-19 immunological features using single-cell RNA sequencing. *Innovation* **3**(5): 100289. <https://doi.org/10.1016/j.xinn.2022.100289>.
209. Li, T., Huang, T., Guo, C., et al. (2021). Genomic variation, origin tracing, and vaccine development of SARS-CoV-2: A systematic review. *Innovation* **2**(2): 100116. <https://doi.org/10.1016/j.xinn.2021.100116>.
210. Ramírez-Flores, C.J., and Knoll, L.J. (2021). Human intestinal enteroids as a model for SARS-CoV-2 infection and antiviral drug screening. M.J. Evans, ed. **17**(11): e1010080. <https://doi.org/10.1371/journal.ppat.1010080>.
211. Bartfeld, S. (2021). Organoids as host models for infection biology – a review of methods. *Exp. Mol. Med.* **53**: 1471–1482. <https://www.nature.com/articles/s12276-021-00629-4>.
212. Huang, Y., Huang, Z., Tang, Z., et al. (2021). Research progress, challenges, and breakthroughs of organoids as disease models. *Front. Cell Dev. Biol.* **9**: 740574. <https://doi.org/10.3389/fcell.2021.740574>.
213. Blutt, S.E., and Estes, M.K. (2022). Organoid models for infectious disease. *Annu. Rev. Med.* **73**(1): 167–182.
214. Masmoudi, F., Santos-Ferreira, N., Pajkrt, D., et al. (2023). Evaluation of 3D human intestinal organoids as a platform for EV-A71 antiviral drug discovery. *Cells* **12**(8): 1138. <https://doi.org/10.3390/cells12081138>.
215. Ramezankhani, R., Solhi, R., Chai, Y.C., et al. (2022). Organoid and microfluidics-based platforms for drug screening in COVID-19. *Drug Discov. Today* **27**(4): 1062–1076. <https://doi.org/10.1016/j.drudis.2021.12.014>.
216. Bartfeld, S. (2016). Modeling infectious diseases and host-microbe interactions in gastrointestinal organoids. *Dev. Biol.* **420**(2): 262–270. <https://doi.org/10.1016/j.ydbio.2016.09.014>.
217. Duque-Correa, M.A., Maizels, R.M., Grenic, R.K., et al. (2020). Organoids – new models for host–helminth interactions. *Trends Parasitol.* **36**(2): 170–181. <https://doi.org/10.1016/j.pt.2019.10.013>.
218. Thompson, R.E., Bouma, G.J., and Hollinshead, F.K. (2022). The roles of extracellular vesicles and organoid models in female reproductive physiology. *Int. J. Mol. Sci.* **23**(6): 3186. <https://doi.org/10.3390/ijms23063186>.
219. (2022). S18.3: Building organoid and microfluidic models to identify toxicant risks to female reproduction. *Am. J. Reprod. Immunol.* **87**(S1): 72–73. https://doi.org/10.1111/aji.90_13546.
220. Alzamil, L., Nikolakopoulou, K., and Turco, M.Y. (2021). Organoid systems to study the human female reproductive tract and pregnancy. *Cell Death Differ.* **28**(1): 35–51. <https://doi.org/10.1038/s41418-020-0565-5>.
221. Yang, L., Semmes, E.C., Ovies, C., et al. (2021). Innate immune signaling in trophoblast and decidua organoids defines differential antiviral defenses at the maternal-fetal interface. Preprint at BioRxiv. <https://doi.org/10.1101/2021.03.29.437467>.
222. Brazovskaja, A., Treutlein, B., and Camp, J.G. (2019). High-throughput single-cell transcriptomics on organoids. *Curr. Opin. Biotechnol.* **55**: 167–171. <https://doi.org/10.1016/j.copbio.2018.11.002>.
223. Hof, L., Moreth, T., Koch, M., et al. (2021). Long-term live imaging and multiscale analysis identify heterogeneity and core principles of epithelial organoid morphogenesis. *BMC Biol.* **19**(1): 37. <https://doi.org/10.1186/s12915-021-00958-w>.
224. Yang, R., Du, Y., Kwan, W., et al. (2023). A quick and reliable image-based AI algorithm for evaluating cellular senescence of gastric organoids. *Cancer Biol. Med.* **20**: 519–536. Published Online. <https://doi.org/10.20892/j.issn.2095-3941.2023.0099>.
225. Leung, C.M., De Haan, P., Ronaldson-Bouchard, K., et al. (2022). A guide to the organ-on-a-chip. *Nat Rev Methods Primer* **2**(1): 33. <https://doi.org/10.1038/s43586-022-00118-6>.
226. Guenet, O.T., Geiser, T., and Berthiaume, F. (2020). Clinically relevant tissue scale responses as new readouts from organs-on-a-chip for precision medicine. *Annu. Rev. Anal. Chem.* **13**(1): 111–133. <https://doi.org/10.1146/annurev-anchem-061318-114919>.
227. Teriyapirom, I., Batista-Rocha, A.S., and Koo, B.K. (2021). Genetic engineering in organoids. *J. Mol. Med.* **99**(4): 555–568. <https://doi.org/10.1007/s00109-020-02029-z>.
228. Lim, S.W., Fang, X., Cui, S., et al. (2023). CRISPR-Cas9-Mediated correction of SLC12A3 gene mutation rescues the Gitelman's disease phenotype in a patient-derived kidney organoid system. *Int. J. Mol. Sci.* **24**(3): 3019. <https://doi.org/10.3390/ijms24033019>.
229. Li, X., Fu, G., Zhang, L., et al. (2022). Assay establishment and validation of a high-throughput organoid-based drug screening platform. *Stem Cell Res. Ther.* **13**(1): 219. <https://doi.org/10.1186/s13287-022-02902-3>.
230. Wu, X., Jiang, D., Yang, Y., et al. (2023). Modeling drug-induced liver injury and screening for anti-hepatofibrotic compounds using human PSC-derived organoids. *Cell Regen.* **12**(1): 6. <https://doi.org/10.1186/s13619-022-00148-1>.
231. Fleck, J.S., Jansen, S.M.J., Wollny, D., et al. (2021). Inferring and perturbing cell fate regulomes in human cerebral organoids. Preprint at BioRxiv. <https://doi.org/10.1101/2021.08.24.457460>.
232. Vento-Tormo, R. (2023). Single-cell omics meets organoid cultures. *Nat. Rev. Genet.* **24**(8): 492. <https://doi.org/10.1038/s41576-023-00622-9>.
233. Yin, Y., Liu, P.Y., Shi, Y., et al. (2021). Single-cell sequencing and organoids: a powerful combination for modelling organ development and diseases. In *Reviews of Physiology, Biochemistry and Pharmacology*, S.H.F. Pedersen, ed. (Springer International Publishing), pp. 189–210. https://doi.org/10.1007/112_2020_47.
234. Jagadeesh, K.A., Dey, K.K., Montoro, D.T., et al. (2022). Identifying disease-critical cell types and cellular processes by integrating single-cell RNA-sequencing and human genetics. *Nat. Genet.* **54**(10): 1479–1492. <https://doi.org/10.1038/s41588-022-01187-9>.
235. Jin, F., Liu, M., Zhang, D., et al. (2023). Translational perspective on bone-derived cytokines in inter-organ communications. *Innovation* **4**(1): 100365. <https://doi.org/10.1016/j.xinn.2022.100365>.
236. Du, X., Chen, Z., Li, Q., et al. (2023). Organoids revealed: morphological analysis of the profound next generation in-vitro model with artificial intelligence. *Biodes. Manuf.* **6**(3): 319–339. <https://doi.org/10.1007/s42242-022-00226-y>.
237. Kassib, T., Hernandez-Gordillo, V., Langer, R., et al. (2019). OrgaQuant: human intestinal organoid localization and quantification using deep convolutional neural networks. *Sci. Rep.* **9**(1): 12479. <https://doi.org/10.1038/s41598-019-48874-y>.
238. Kegeles, E., Naumov, A., Karpulevich, E.A., et al. (2020). Convolutional neural networks can predict retinal differentiation in retinal organoids. *Front. Cell. Neurosci.* **14**: 171. <https://doi.org/10.3389/fncel.2020.00171>.
239. Bein, A., Shin, W., Jalili-Firoozinezhad, S., et al. (2018). Microfluidic organ-on-a-chip models of human intestine. *Cell. Mol. Gastroenterol. Hepatol.* **5**(4): 659–668. <https://doi.org/10.1016/j.jcmgh.2017.12.010>.
240. Valiei, A., Aminian-Dehkordi, J., and Mofrad, M.R.K. (2023). Gut-on-a-chip models for dissecting the gut microbiology and physiology. *APL Bioeng.* **7**(1): 011502. <https://doi.org/10.1063/5.0126541>.
241. Kasendra, M., Tovaglieri, A., Sontheimer-Phelps, A., et al. (2018). Development of a primary human small intestine-on-a-chip using biopsy-derived organoids. *Sci. Rep.* **8**(1): 2871. <https://doi.org/10.1038/s41598-018-21201-7>.
242. Campisi, M., Shin, Y., Osaki, T., et al. (2018). 3D self-organized microvascular model of the human blood-brain barrier with endothelial cells, pericytes and astrocytes. *Biomaterials* **180**: 117–129. <https://doi.org/10.1016/j.biomaterials.2018.07.014>.
243. Hajal, C., Offeddu, G.S., Shin, Y., et al. (2022). Engineered human blood–brain barrier microfluidic model for vascular permeability analyses. *Nat. Protoc.* **17**(1): 95–128. <https://doi.org/10.1038/s41596-021-00635-w>.
244. Tian, M., Ma, Z., and Yang, G.Z. (2024). Micro/nanosystems for controllable drug delivery to the brain. *Innovation* **5**(1): 100548. <https://doi.org/10.1016/j.xinn.2023.100548>.
245. Pereira, A., Garcia, J.W., and Muotri, A. (2023). Neural stimulation of brain organoids with dynamic patterns: a sentiomics approach directed to regenerative neuromedicine. *NeuroSci* **4**(1): 31–42. <https://doi.org/10.3390/neurosci4010004>.
246. Serruya, M.D. (2017). Connecting the brain to itself through an emulation. *Front. Neurosci.* **11**: 373. <https://doi.org/10.3389/fnins.2017.00373>.
247. Kagan, B.J., Kitchen, A.C., Tran, N.T., et al. (2022). In vitro neurons learn and exhibit sentence when embodied in a simulated game-world. *Neuron* **110**(23): 3952–3969.e8. <https://doi.org/10.1016/j.neuron.2022.09.001>.
248. Smirnova, L., Caffo, B.S., Gracias, D.H., et al. (2023). Organoid intelligence (OI): the new frontier in biocomputing and intelligence-in-a-dish. *Front. Sci.* **7**: 1017235. <https://doi.org/10.3389/fsci.2023.1017235>.

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AUTHOR CONTRIBUTIONS

X. Han, C.D., X. He, and X.C. provided direction and guidance throughout the preparation of this manuscript. X. Han, C.C., and W.D. collected and interpreted studies and were major contributors to writing and editing of the manuscript. B.F., Y.S., L.L., C.W., J.Z., M.R., J.L., H.H., and X.L. participated in discussions, language editing, and manuscript revision. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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