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Next-Generation Organoids: Redefining Human Disease Modeling and Drug Discovery

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Abstract: Organoid technology models human tissues efficiently in vitro, preserving organ properties and functions. By recapitulating tissue architecture and physiology, organoids advance our understanding of human embryogenesis, tissue development, and disease. When combined with engineered platforms, patient-derived organoids transform biomedical research, accelerating preclinical assessments of candidate drugs. Patient-derived tumor organoids stand out as tools for exploring tumor-immune interactions and personalized medicine. Emerging application areas include genome and epigenome editing, antibody discovery, and transcriptomic analyses . The brief summarizes organoid technology, explores advances in organoid types and culture methodologies, and examines applications in disease modelling and drug discovery. Next-generation organoids combined with engineered substrates enable artificial tissues at centimetre scale with functional vasculature,

facilitating large-scale production for a broad spectrum of human tissues. The review identifies key challenges and highlights future trends in the field.

1. Introduction to Organoids

Organoids are defined as intricate three-dimensional (3D) structures that are grown from stem cells in vitro. These organoids consist of various organ-specific cell types that intricately self-organize into structures that closely resemble parts of an organ or even the entire organ itself, much like the processes observed during early embryonic formation and development. Organoids derived from human pluripotent stem cells, as well as adult stem cells, have convincingly demonstrated their immense potential as relevant and valuable biological systems for studying various aspects of human development and effectively modeling a wide range of diseases. Furthermore, they are being utilized with increasing frequency for drug discovery purposes and innovative cell replacement strategies, showcasing their versatility and importance in contemporary biomedical research. [1][2]

2. Historical Overview of Organoid Technology

The organoid field has witnessed several milestones over recent decades. The self-organization of a single cell into full, complex, and functional human organs in vitro — signifying the fruition of the organoid concept — was first realized with the reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) for intestinal organoid generation [3].

Earlier, intestinal organoids derived from Lgr5 stimulated both developmental and cancer pathways by recapitulating the intestinal crypt–villus organ architecture [2]. Brain organoids developed through spontaneous self-organization of ultrasound-guided microdissected anlagen enabled studies on human brain development.

Ab-initio methods facilitated the generation of regionally restricted human forebrain organoids. Brain organoids subjected to active mechanical reconfiguration of embryoid-body patterns consisting of ternary promoters produced an integrated specification approach for more forebrain organoids.

Pancreatic differentiation of iPSCs effectively utilized phosphate-functionalized biomaterials to significantly enhance the development of three-dimensional pancreatic organoids, which in turn improved long-term hormone secretion for an impressive duration of up to 42 days. Furthermore, hepatobiliary organoids showed notable capabilities in biliary regeneration and addressing fibrosis issues, thereby advancing the efforts in liver disease modeling and providing valuable insights into treatment methodologies.

3. Types of Organoids

Organoids are truly remarkable three-dimensional miniaturized tissue cultures that are meticulously propagated in vitro using a variety of different types of cells, which can include patient-derived primary cells, embryonic stem cells, or even induced pluripotent stem cells (iPSCs). These innovative structures offer an exciting opportunity for the development of a complex multi-cellular, organotypic micro-anatomy, which effectively recapitulates and very closely mimics the individual biology of the specific source patient or donor tissue. This extraordinary ability to replicate specific biological characteristics makes organoids an incredibly powerful and versatile tool in the realm of scientific research. They have been extensively described and developed across a wide range of organs, including, but not limited to, the pancreas, intestines, brain, and liver. This significant advancement has substantially enhanced our understanding of organ-specific biology and disease modeling, providing insights

that are invaluable in the fields of medicine and therapeutic interventions. [2]

3.1. Intestinal Organoids

These stem cell-derived intestinal organoids are pivotal in studying intestinal physiology and pathology [4]. Under culture conditions simulating the in vivo intestinal epithelial growth environment, they faithfully replicate self-renewal processes and facilitate investigations into microbiota–intestinal epithelium–immune interactions. The core organoid culture technology encompasses three-dimensional (3D) culture, detection, identification, stem cell isolation, organoid extraction, and organoid preservation, augmented by emerging methods such as 3D bioprinting, organoid microarrays, and gene editing, which broaden research capabilities and applications. Intestinal organoid models effectively recapitulate developmental milestones of the human intestinal epithelium and enable in vitro exploration of paracellular permeability. Their utility extends to modeling infectious diseases and colorectal cancer initiation; notably, patient-derived bang-sensitive (bs) organoids use RNA sequencing to elucidate mechanisms underlying drug resistance. The addition of exogenous growth factors and the implementation of bioengineering techniques further enhance organoid models, promoting robust support for large-scale research and accelerating drug discovery [2].

3.2. Brain Organoids

Brain organoids have recently emerged as a widely used biological tool for modeling neurological diseases. Originally generated as unguided whole-brain organoids, more advanced systems producing vascularized region-specific organoids such as cortex, midbrain, hippocampus, and cerebellum have now become feasible. Brain organoids enabled the in vitro recapitulation of biological events relevant to human brain development, and have the potential to elucidate mechanisms underlying the pathogenesis of neurodevelopmental psychiatric disorders and neurodegenerative diseases. However, the culture times currently feasible in vitro do still not allow the development of fully mature brain structures, and are therefore more relevant for the study of biological events occurring during embryonic human brain development. Nevertheless, brain organoids promise to reveal previously inaccessible aspects of the human brain and neurological diseases, and have the potential to become invaluable models for better understanding the fundamental biology of brain development and function. [5][6][7]

3.3. Liver Organoids

Organoids derived from the liver help uncover mechanisms of liver development, regeneration, disease, and various liver-specific disorders. Organoid models can be generated from both primary tissue and pluripotent stem cells. PSC-derived organoids mimic developmental processes, whereas tissue-derived organoids recapitulate the regeneration programs of adult liver cells. Hepatic organoids contain differentiated hepatocytes and generate multiple lineages, allowing both the study of tissue specification and the investigation of monogenic liver diseases such as Alagille syndrome and ciliopathies.

Human hepatocellular organoids possess the remarkable ability to be differentiated into cholangiocytes, thereby effectively modeling a variety of cholangiopathies, including cirrhosis and cystic fibrosis. These cholangiocyte organoids display significant stem cell plasticity, which empowers them to differentiate into hepatocytes. This characteristic is crucial as it allows for the potential to halt the progression of liver failure in a murine model following successful transplantation. Moreover, hepatic organoid models have demonstrated their usefulness in investigating conditions such as steatohepatitis, alcoholic liver damage, and diverse forms of viral hepatitis. They also play a pivotal role in the study of infectious diseases, including yellow fever and malaria associated with Plasmodium. Taken together, liver organoids reveal immense potential across a broad spectrum of research areas, encompassing development, regeneration, and a multitude of diseases, while also addressing fundamental questions that arise in the study of liver biology. [8][9][10]

3.4. Pancreatic Organoids

Pancreatic organoids have emerged as a vital translational platform bridging the gap between two-dimensional in vitro cell lines and in vivo animal models, thereby advancing investigations into pancreatic cell biology [11]. Utilizing an optogenetic approach implemented in pancreatic islet organoids differentiated from human pluripotent stem cells, researchers have achieved controlled insulin release and demonstrated human C-peptide secretion upon transplantation into diabetic mice. Furthermore, a microfluidic multi-organoid system enabling the co-culture of liver and islet organoids from human induced pluripotent stem cells facilitates metabolic interaction analyses over extended periods; islet organoids maintained in this configuration exhibit glucose-stimulated insulin secretion, while liver organoids display increased glucose consumption when exposed to islet-derived factors [12]. Generation methodologies encompass sources such as human pluripotent stem cells, embryonic stem cells, and various human tissues. Incorporating human amniotic epithelial cells into pancreatic organoids enhances engraftment efficiency and functional outcomes in diabetic animal models. Patient-derived pancreatic tumor organoids replicate tumor histopathology and transcriptomic profiles, serving as robust preclinical platforms for long-term drug screening, thereby informing personalized therapeutic strategies and enabling serial assessment of chemotherapy response dynamics.

4. Organoid Culture Techniques

To fully realize their potential, organoids require 3D culture protocols incorporating appropriate biomaterials to sustain growth and differentiation [2]. Various systems are available, including extracellular matrix (ECM)-based basement membrane extracts, scaffolds formed from biological or synthetic materials, and combinations where synthetic scaffolds are coated with ECM [13]. The choice of biomaterial influences organoid size, shape, and functionality. Matrix components provide critical biochemical signals, while mechanical properties contribute to viscoelastic support and mechanotransduction.

Existing culture procedures often lack precise control over physicochemical factors such as oxygen tension, pH, temperature, nutrient supply, and metabolite accumulation. Additionally, current protocols limit the complexity of organoid systems, hindering the inclusion of various cell populations needed to replicate fully the structural and functional heterogeneity of the corresponding organ. Addressing these challenges is fundamental to both the modeling of human organs and the examination of disease processes. Organoids cultured using well-defined microenvironments and optimized platforms will not only retain but may enhance their physiological relevance, becoming indispensable research tools with significant therapeutic promise.

4.1. 3D Cell Culture Methods

In the human body, interactions among cells and the extracellular matrix are fundamental over the temporal scale of a lifetime. Biological cell functionality and structure evolve during development, and the expression of specific proteins governs specialized cellular roles, such as insulin secretion or electrical signal transmission. Studies indicate that cellular functionality cannot be fully achieved in two-dimensional (2D) culture systems due to the absence of normal mechanical and functional stimuli. Consequently, 2D monolayers are insufficient to replicate, for example, the functionality of intestinal cells, as referenced in reports by [14] and [15]. Consequently, researchers have developed three-dimensional (3D) culture methods, categorized as scaffold-based or scaffold-free approaches, to better mimic in-vivo tissue architecture.

4.2. Biomaterials in Organoid Culture

Organoids derive from pluripotent stem cells (PSCs) or adult/somatic stem cells (ASCs) and are often cultured in biomimetic materials that support the three-dimensional (3D) spatial architecture conducive to self-organization and self-renewal. Three-dimensional organoid systems are generally classified into two categories: embedded cultures for intestinal, optic-cup,

and neural organoids, and suspended cultures for cerebral and bronchial organoid budding. Commonly applied non-cellular supports include animal-derived scaffolds such as Matrigel and collagen, along with synthetic hydrogels. Polysaccharide hydrogels play an important role as biological materials in the encapsulation and culture of stem cells for organoid formation.

Hydrogels provide optimal water content, facilitating the diffusion of nutrients, oxygen, and bioactive agents, which is crucial for copy number uniformity, morphogen supply, and cell viability. Nonetheless, the use of Matrigel, containing more than 60 biochemical components, restricts functional investigations of cellular microenvironmental cues due to its complexity and variability. This challenge has catalyzed the development of synthetic hydrogels with adjustable biochemical and mechanical properties designed to support stem cell growth and differentiation for organoid formation. Notwithstanding these innovations, existing synthetic hydrogels must ultimately demonstrate compatibility with other target organ-in-chip models to enable modular multi-organoid-on-chip platforms.

5. Applications in Disease Modeling

Organoids emulate tissue conditions more faithfully than tumor-sphere cultures, facilitating more accurate drug discovery [3]. As a three-dimensional (3D) culture system, organoids can be reconstructed into appropriate 3D structures during in vitro culture. Although different methodologies are indicated, the advances of recent techniques have improved organoid culture and enabled applications in modeling brain, retinal, liver, kidney, pancreas, and intestinal diseases. Organoids are widely utilized to recapitulate human disease microenvironments in diverse areas, including cancer, neurodegenerative disorders, and infectious diseases. These models retain the architecture of the original tissues and enable high-throughput drug screening with chemical libraries, providing a strong platform to identify pathways associated with diseases and drug responses.

Ascidians have long served as models to investigate the mechanisms of development, regeneration, and immunology. Current culture approaches establish ascidian organoid models from whole embryos and adult intestine cells. Organoids derived from embryos mimic developing intestine-like organs and retain the capacity for differentiation. Similarly, organoids from adult intestine cells reproduce the original tissue’s cellular assembly, retaining functionality [2]. Moreover, intestinal organoids continue to proliferate as mesenchymal-retaining organoids but keep the mesenchymal compartment as an integral part of the culture matrix. These observations strengthen the hypothesis of an in vivo-like state, extending possible applications to model ascidian intestine physiology during long-term cultivation, from development to immune-endocrine responses.

5.1. Cancer Research

During a normal colonic epithelial regeneration process, genetic insults accumulated throughout the lifecycle of focally damaged epithelial cells drive the cancerous transformation of patients in the colorectal tract [16]. Cancer treatment and drug screening suites can be greatly expanded from limited starting material, enabling fine-tuned analyses of intestinal stem cell behavior; drug screening; disease modeling; and genetic screening [17]. The ability to generate and cryopreserve biobanks of healthy and diseased living human colon organoids from individuals provides a renewable resource for physiological studies and drug screening. Carcinogenesis can be modelled by mutating a predefined set of driver genes in wild-type human colon organoids, with the CRISPR–Cas9 system allowing precise manipulation of virtually every gene combination [18].

5.2. Neurodegenerative Diseases

Neurodegenerative diseases feature prominently among the disorders modeled using organoid technology [19]. Their insidious nature makes them difficult to diagnose and increasingly urgent to treat. Building upon a foundation of induced pluripotent stem cell (iPSC) approaches, next-

generation brain organoids enable investigations into the mechanisms, pathways, genetics, and development of neurotransmitters associated with major late-onset disorders such as Alzheimer's (AD) and Parkinson's (PD) diseases. Both exhibited hallmarks can be recapitulated in these models, opening the door to future preventative therapeutics and personalized medicine. These technologies thereby offer a means to evaluate the toxicity and efficacy of drug candidates in a patient-specific context. Additional neurodegenerative disorders modeled in human brain organoids, such as Huntington's disease, frontotemporal dementia, and prion diseases, underscore human brain organoids as a viable option for urgent development of drug discovery tools and therapeutic strategies.

5.3. Infectious Diseases

Many of the most common and damaging human diseases are caused or driven by infectious pathogens. Infectious diseases, which affect millions annually, cost billions of dollars in annual losses, and are a major source of human pain and suffering. Live human tissue infection models are obstructed by a lack of reprogramming technologies and by restrictions on human tissue access; in response, organoids afford a valuable means to investigate human infectious diseases. Infectious diseases arise from the introduction and establishment of a pathogen that triggers a clinically apparent physiologic response and sometimes downstream clinical pathology. Disease-causing pathogens span viruses (e.g., Retroviridae and Adenoviridae), bacteria (e.g., E. coli and Salmonella), and parasites (e.g., Giardia and Plasmodium). Organoids permit invasion and persistence of pathogens on nearly all organ systems, providing an ideal platform to uncover essential aspects of the infectious life cycle. The complex architecture, organ-specific receptors, and transcriptional signature inherent in organoids facilitate microbe-host interactions.

3D models for human infectious diseases are still in their infancy. Preliminary reports illustrate the potential for organoids to capture important relationships between human tissue and pathogens. Scientists investigating the Zika virus find evidence of microcephaly development in infected brain-like organoids [2]. Reports also evaluate Clostridium difficile infection in human intestinal organoids. Unfortunately, the absence of vascularization or immune cells limits the interpretation of organoid data. Defective, immature differentiation, and poor biophysical properties that inadequately support infection dynamics detract from model accuracy [20]. Several avenues promise to address these deficits. The addition of native immune cells triggers a physiologically representative immunological response. Vascularization exposes the system to shear forces, gradients, and other microenvironmental stimuli. Finally, cough chambers permit an infection-mimicking bolus reminiscent of in vivo exposure. Several laboratories assemble co-culture platforms integrating stem-cell-, cancer-, and primary-derived organoids with native tissue-resident immune cells. Presented with relevant inflammatory stimuli, intrinsic tissue-resident immune cells contact the organoid, through which they infiltrate and infiltrate themselves within the epithelium. These preliminary platforms readily incorporate innate immune components; however, the absence of blood precludes studies on adaptive immune response and rolling/circulating recruitment that encompasses the greater systemic infection and recruitment behavior. Many researchers develop microfluidic organoid-on-a-chip platforms with vascularization; currently, these platforms are limited to stem-cell-derived organoids, reducing the diversity of human tissues that can be studied. Immune enhancements that incorporate blood into such vascularized platforms will undoubtedly reduce this hurdle. Microbial co-culture presents another dimension: commensal microbiota figure prominently in processes underpinning human health and disease. Organoid microfluidic platforms that recapitulate essential aspects of the human microbiome permit mechanistic studies.

6. Organoids in Drug Discovery

Organoid technology offers the opportunity to make the drug discovery process more efficient and reduce the failure rate in the clinical phase. Indeed, the use of animal models has become inefficient in certain cases, because only very complex animals seem to be able to reproduce

complex phenotypes. However, the culture of complex tissues from these animals is still hard. Drug screening involves many stages and enormous investments. Therefore, it is worthwhile to put efforts into making disease models as close as possible to human pathophysiology. Over the last years, many efforts have been devoted to refining transcriptomic techniques, and gene expression and regulation can be analyzed during organoid culture and differentiation.

Strategies implementing virus infection in organoids represent powerful tools for screening drugs able to inhibit such processes. High-throughput screens in cancer organoids represent a valuable alternative to identify gene vulnerabilities and phenotypic drug responses. Moreover, drug screening on patient-derived cells can inform personalized clinical strategies. The development of high-throughput screening represents an important step towards the integration of drug discovery with next-generation organoid models. Ideally, drug treatment on organoids should be combined with more complex techniques, such as biomechanical studies, to maximize the benefits that organoid culture can offer. [21][22][23]

6.1. High-Throughput Screening

High-throughput screening is a powerful technique that aims to rapidly categorize bioactive compounds and characterize molecular targets through large-scale testing of chemical libraries. Screening methods include transgenic and chemical mutagenesis, RNA interference (RNAi), small-molecule libraries, and yeast two-hybrid approaches [24]. Sometimes it remains unclear if the effects are due to direct action on the target or through secondary mechanisms. To address this, a high-throughput, automated, programmable microfluidic platform has been developed for 3D tumor organoid culture and screening, enabling dynamic and combinatorial drug treatments along with real-time, non-invasive monitoring. Validation studies on human pancreatic tumor organoids revealed patient-based variations in response and demonstrated that temporal modulation of drug treatments can outperform constant-dose monotherapies or combinations [15]. Primary human organoids derived from metastases maintain phenotypic and mutational profiles after extended passaging and cryopreservation, facilitating their use in drug discovery. Many organoid culture methods are currently inefficient or costly, rendering them unsuitable for high-throughput applications. A novel approach combines cell-repellent surfaces with bioprinting and magnetic force to produce uniform organoids in standard culture plates. Screening anticancer drugs against primary pancreatic cancer cells and associated stromal fibroblasts using this method, alongside parallel two-dimensional assays, highlights the enhanced relevance of three-dimensional formats for oncology drug discovery.

6.2. Personalized Medicine

The ability to rapidly generate patient-derived organoids allows for the extraction of biopsy samples to produce organoids that can be analyzed by deep-sequencing and phenotyped in a variety of assays to determine the most suitable treatment regimens [16]. While organoids could be applied to test any form of personalized therapy, they hold particular promise in the field of oncology, where patient-derived xenografts (PDXs) are used extensively as testbeds for potential treatments. However, not all tumour tissues are amenable to PDX engraftment, and the process of engraftment and expansion can be lengthy and costly, especially when long-term expansion and passaging are required. Organotypic models provide a promising alternative, and their ability to grow across many tumour types, coupled with rapid expansion, improved scalability and a homology to the parental tumour, make them an ideal platform for testing personalised treatment regimens [15].

7. Organoid Models of Human Development

An increasing number of organoid systems provide a novel platform for modeling human development through experimentation on adult tissue resections or pluripotent cell derivatives. Because human development is often longer, more hands-off, and tissue specific than in model animals, its study in human samples is especially promising. For example, intestinal organoids

generate cell diversity and niche architecture from a single jejunal crypt in vitro, offering a model for committed stem cell populations, apoptosis-driven villus remodeling, and Wnt-driven crypt expansion. Brain organoids enable interrogation of developmental processes such as neurogenesis and cortical folding. Recent studies reveal morphogen patterning and colonic specification within human gastrointestinal organoids [2]. Organoids therefore help to bridge development and disease, given that the epigenetic and transcriptional mechanisms associated with embryonic stem cells frequently become reactivated in cancers [1].

8. Ethical Considerations in Organoid Research

Ethical debate remains pertinent as organoid technology moves toward clinical translation and as its use in modeling development becomes increasingly prevalent. Unlike stem cell-derived models, organoid derivation directly consumes a patient's tissue sample, which was originally collected for diagnostic or therapeutic purposes, implying that re-consent may be required when establishing a living tissue model.

The ethical literature on clinical applications of organoid technology is most focused on two applications: personalized medicine (in vitro) and transplanting organoids in humans (in vivo). Patient-derived organoids facilitate drug testing for personalized medicine, predicting the effectiveness of individual treatments and potentially reducing exposure to ineffective drugs with known side effects. For example, cystic fibrosis treatment with ivacaftor can be prescribed only when corresponding organoids from the patient show a positive response. The model further informs the selection of suitable drug dosages [25].

Maintaining the patient-organoid linkage to protect privacy constitutes a major concern, as does the high cost and time required to generate and maintain organoids, limiting accessibility. Difficulty in translating laboratory responses to clinical outcomes raises skepticism about the additional clinical value of organoids, given their restricted capacity to predict whole-body responses. Validation remains a challenge on both technical and ethical grounds, particularly for rare diseases, where single-patient trials have been proposed to assess safety and efficacy.

9. Challenges and Limitations

Organoids offer a promising platform to study human development, disease and to accelerate drug development, but several challenges remain [2]. The size of organoids is limited by nutrient supply, as lacking vasculature, their development depends on diffusion, leading to necrosis in thicker tissues like the brain. Efforts focus on improving nutrient supply and vascularization, for example by implantation into angiogenic sites in rodents enabling blood perfusion. Vascularization approaches reveal that human-specific metabolites can be detected in rodent blood and vascularized brain organoids can attract host microglia. Fluid flow applications to kidney organoids promote endothelial network formation and tissue maturation [26]. Scalability remains a challenge due to the complex, labour-intensive nature of assays and the need for larger culture vessels, prompting development of bioreactors like Spin-Ω. The self-organisation of organoids introduces unpredictability in tissue identity and configuration, necessitating careful selection and analysis, supported by bioengineering methods using scaffolds and micropatterned substrates. Large-scale drug testing requires scaling up organoid production, an approach currently explored by the pharmaceutical industry. Organoids can also be combined with existing models, such as isolating cells for 2D culture or employing organ-on-a-chip and microfluidics techniques to study cellular diversity and fluid dynamics. Organoids are likely to complement, rather than replace, traditional animal models in drug development. Liver organoids and liver-on-a-chip models are increasingly used for drug metabolism testing, aligning with FDA requirements for metabolite demonstration. A long-term goal is using organoids for cell replacement or whole-organ transplantation to address organ shortages, although significant technological advances are still required.

9.1. Technical Challenges

Several technical challenges limit organoid applications for modeling disease and screening drugs, underscoring priority targets for improvement.

Manufacturing organoids at scale remains a central hurdle. Conventional protocols remain labor-intensive and difficult to automate for high-throughput workflows [2]. The miniaturized, bioreactor-based culture systems required to meet screening demands continue to evolve. Variability between differentiation batches compromises repeatability, further complicating efforts to assemble well-defined experimental cohorts.

The physical size of organoids is restricted by nutrient and oxygen diffusion through 3D matrices. A vasculature network would dramatically increase achievable culture dimensions and incubate a wider range of cell populations and spatial configurations, enabling enhanced recapitulation of native organ biochemistry and signaling. Engineered vasculature constructs have shown partial restoration of growth and maturation capacity, opening a promising avenue. Beyond reliable coupling of cellular cohorts with supporting scaffold elements, integrating microfabricated infrastructures as platforms for biochemical delivery, biomechanical stimulation, and biophysical monitoring would also expand the modeling landscape [13]. In particular, the ability to programmatically deliver morphogen pulses during embryogenesis-scale treatments would allow more complex organogenic architectures.

Ultimately, modern organoid technologies provide precise cellular environments that control microstructure complexity. The next frontier lies in the selective integration of this platform with complementary technologies, such as complementary self-organization principles, engineered tissue building blocks, perfusable microfluidics, and robotic automation — configurations that would enrich such fields as human disease modeling and drug discovery for the foreseeable future.

9.2. Biological Variability

Besides the technical challenges inherent to the protocols, organoids are also subject to biological variability. The use of PSC- or tissue-derived organoids entails variation between tissue donors and between different PSC lines, while iPSC reprogramming and gene editing can also introduce variability. The spontaneous self-organization process underlying organoid formation induces variability both between organoids and within individual ones, imposing the need for careful selection of the organoids to be employed in downstream procedures. Consequently, multiple organoids need to be analysed. To mitigate batch-to-batch variability, researchers have developed bioengineering tools such as scaffolds with well-defined architectures and micropatterned substrates to guide organoid development towards increased reproducibility [2]. In the context of drug discovery, organoids are frequently used alongside traditional two-dimensional cultures and organ-on-a-chip approaches, which offer scalability and enhance cellular diversity. Organoids are instrumental for drug metabolism studies, particularly hepatic models employed in the detection of FDA-required intermediate and secondary metabolites. Long-term strategies envision the application of organoid technology for cell replacement therapies and organ transplantation to address the scarcity of donor tissues, although significant technological progress remains necessary [1].

10. Future Directions in Organoid Research

Human organoids are three-dimensional, multicellular, stem cell-derived structures that recapitulate native organs through cell–cell self-organization. They have been widely used in human disease modeling and drug discovery. These next-generation organoids are revolutionizing these fields through integration with emerging technologies such as genome editing, single-cell RNA sequencing, bioinformatics, and machine learning. By mimicking human pathophysiology more accurately and offering higher-throughput and personalized platforms, organoids have transformed our understanding of developmental and disease

mechanisms. They open new avenues for drug and vaccine development and enable precision and translational medicine. Nonetheless, ethical concerns regarding the use of human tissues and the origin of stem cells must be carefully considered to ensure responsible organoid research.

From a technical perspective, significant variability exists in organoid batch production and protocols across laboratories. Resolving these limitations will greatly accelerate the development of organoid applications in academia and the pharmaceutical industry, yielding new drugs and treatments for previously incurable diseases and narrowing the gap between bench and bedside. Looking ahead, future studies will likely employ machine learning to integrate multi-omics, clinical data, and other essential information in the development of organoid-based disease models and drug-discovery platforms, thereby further enhancing the scope of organoid research. [27][28][29]

10.1. Integration with Artificial Intelligence

Organoids are remarkable and sophisticated, self-organizing three-dimensional (3D) multicellular tissue models that are derived from various primary tissues, pluripotent stem cells (PSCs), or induced pluripotent stem cells (iPSCs). These innovative structures are particularly significant because they effectively recapitulate specific organ functions along with complex three-dimensional (3D) architectures that resemble those found in natural organs. Their unique ability to bridge the gap between conventional two-dimensional (2D) cell culture systems and actual in vivo organs makes organoids exceptionally valuable as platforms for studying both normal physiology and the underlying mechanisms of pathophysiology. Thus, they offer a more accurate representation of the biological processes compared to traditional methods. Although the field is still in relatively early stages, the integration of artificial intelligence (AI) with organoid research is poised to revolutionize the landscape of human disease modeling. This synergy has the potential to significantly enhance drug discovery processes, ushering in new possibilities for therapeutic development.

10.2. Expansion into Other Organ Systems

The expansion of organoid technology into additional organs has proceeded at a remarkable pace, and organoid models are now not only available for several human organs but are also significantly advancing both basic and translational studies in biomedical research. The extension of mini-gut organoids to various other portions of the intestinal tract shortly after their initial introduction provided an illuminating glimpse of the breadth and versatility of their applicability in scientific investigation. Moreover, lung organoids can be generated from progenitor cells as well as from adult alveolar type 2 cells (AT2s), which serve a vital role as AT2 stem cells in the intact adult lung, highlighting their potential in respiratory research. The availability of refined culture protocols for these two distinctly characterized progenitors has raised an intriguing and fundamental question among researchers: How similar or distinctive are organoids that are generated from developmentally related, but molecularly distinguishable, cell populations? Additionally, organoids of the kidney that are capable of collecting duct tubule formation also provide valuable new insights into the complex process of branching morphogenesis. The ongoing development of human organoid models representing the internal ear, thymus, and ductal pancreatic cancer holds significant promise for not only understanding the intricate development of these organs but also deciphering the origins and mechanisms of associated diseases. Whereas organoid cultures derived from the gut, prostate, and mammary glands lend themselves exceptionally well to the in vitro study of both normal and neoplastic stem cell behavior, lung and brain organoids are paving the way for new and exciting approaches toward fundamental discoveries in both normal and pathological biological processes. This rapid advancement in organoid research represents a paradigm shift with the potential to transform our understanding of human biology and disease at unprecedented levels. [2][13]

11. Case Studies in Organoid Research

Precise in vitro models of normal physiology and disease are essential for understanding drug targets and toxicity in drug discovery. Animal models, primary cells, and in vitro systems have provided important insights into physiology and toxicology, but species-specific differences and throughput limitations have curtailed translation of candidates into humans. Tailored culture systems for specific cell types can partially overcome some limitations, although maintaining fully differentiated cells in vitro remains difficult. High-throughput two-dimensional (2D) models are being improved with increasingly complex culture systems to enhance clinical translation. Nevertheless, for systems biology and personalized medicine, complex and scalable models in highly adaptable formats that allow for simultaneous differentiation and toxicology screening would accelerate development [2]. Organoid cultures are three-dimensional (3D) structures that recapitulate both the cellular and extracellular compositions of progenitor tissues and the spatial physiology of their in vivo counterparts. They retain physiological architecture and cellular heterogeneity absent from 2D systems and provide a tunable microenvironment conducive to long-term growth with limited passaging. Their complexity is balanced by scalability and cost, which places them between immortalized cell lines and organ-on-a-chip or animal systems in terms of screening. Organotypic cultures have been used to establish disease models and generate patient-specific biorepositories, empowering high-throughput screens for cancer therapy [16]. Updated models of a host of diseases are now available, exemplified by the improvements in cholangiocyte organoid development.

11.1. Successful Drug Development Examples

Organoids have been instrumental in developing several drugs, such as the intestinal organoid-based treatments for cystic fibrosis. A biobank of pancreatic cancer organoids has uncovered unique drug sensitivity profiles [26] , while glioblastoma organoids have facilitated personalized strategies for these tumors. Combining genetic and transcriptomic analyses with high-throughput drug efficacy tests has also shown promise in pancreatic ductal adenocarcinoma (PDAC). Brain organoids have been used to identify oxa- and ptero-peptidomimetics that significantly reduce ——————————b1-synuclein-induced neurotoxicity, and organoid platforms have accelerated lead identification for viral infections such as SARS-CoV-2. Mouse mammary tumor organoids have generated mimetics of human drug-resistant breast cancer subtypes. These examples spotlight the transformative potential of next-generation organoids in modeling human disease and facilitating drug discovery.

Human cancer organoids derived from conventional 2D and 3D cell lines support drug screening and basic research but lack the complexity of patient-derived cancer organoids. Such precision tumor models capture inter-tumor heterogeneity and closely represent the tumor phenotype in vitro, serving as valuable complements to patient-derived xenografts with superior genetic stability and expandability. Establishing long-term cultures for various cancers has been achieved, and drug screening has identified therapeutic compounds targeting the ERK pathway in colorectal cancer organoids with distinct mutation profiles. Patient- and disease-specific organoids offer promising tools for drug testing, gene editing, and prognosis evaluation, particularly for malignancies, infections, and developmental disorders. High-throughput screening using organoids is now applied to assess individual variation in drug efficacy and resistance across several cancer types, potentially guiding new pharmaceutical leads. In vitro52organoid models also address issues of animal testing, with intestinal organoids providing a benchmark for studying drug and toxin-induced damage to the intestinal epithelium. Collectively, next-generation organoids promise to redefine in vitro human disease modeling, facilitate precision drug discovery, and transform biomedical research and clinical therapeutics [2] [30].

11.2. Innovative Disease Models

Emerging organoid models are transforming the fields of human development, the modeling of human disease, and drug discovery [2]. Over the past decade, culture systems termed organoids have been developed that use genetically unaltered pluripotent or adult stem cells to form systems that recapitulate the composition, architecture, and physiological aspects of human tissues. These models are now being utilized in cancer, neurodegenerative disorders, and infectious diseases and offer tractable platforms for high-throughput drug screens that may eventually lead to personalized medicine for affected individuals [1]. For these reasons, organoid modelling has become an important tool in studies of early human development, allowing phenomena to be explored in depth and in real time on complex, 3D, and physiologically relevant models.

Organoid culture systems employ 3D culture methodologies and a rich repertoire of biomaterials and scaffolds that support and stimulate cellular self-organization under defined medium conditions. Culture systems can derive 3D brain models that mimic major features of human brain development and disease. Intestinal organoids preserve key aspects of tissue, including characteristic morphology and functional cell types, and recapture organ features that are essential to developmental and adult biology. Liver and pancreatic organoids are likewise capable of faithfully recapitulating key tissue characteristics and represent useful tools for studying human development and disease.

12. Regulatory Framework for Organoid Applications

In vitro culture-based models, which employ various systems such as cells, spheroids, organoids, or organ-on-a-chip methodologies, in addition to whole-organ models, present multiple viable alternatives to animal models in the field of drug discovery. While these innovative approaches do provide enhanced mechanistic insights, they have been identified to produce inconsistent and sometimes contradictory results. This issue particularly arises when examining statistically significant differences in feeding behavior, leading to concerns about their overall suitability and reliability for preclinical studies. The challenge of standardizing novel behavioral assays that are based upon the homeostatic model using zebrafish has demonstrated promise and potential. This strategy could effectively integrate alternative approaches into the mechanistic framework that supports the application of zebrafish in both drug discovery and development processes (RQ1). By refining these assays, researchers hope to overcome current limitations and improve the predictive power of these models in assessing drug efficacy and safety. [31][32][33]

13. Collaboration between Academia and Industry

The emergence of organoids stands as a milestone in biomedical research. Much of the development is anchored in academic research. Nevertheless, progress would be sluggish without the support of industry partners. The combined efforts of academia and industry have been instrumental in broadly establishing the use of organoids as new disease models. Various companies are developing technologies and applications around organoids, representing an important link between academia and industry. An example is a collaboration between University Medical Center Groningen and the Groningen-based company PluriPotent that began in 2017, illustrating the mutual benefits gained from such partnerships [2]. Moreover, the pharmaceutical company Pfizer supports Stem Cell Institute projects focusing on the use of organoids for drug development. This company-affiliated alliance exemplifies the complementary nature of academia and industry in driving organoid research forward. Collaborative partnerships facilitate the two-way flow of knowledge, enabling the identification of areas requiring additional research and the application of novel technologies to better understand various human conditions. The potential for an accelerated shift toward humanized systems is considerable, given that current investment from pharmaceutical companies in organoid technology is only just commencing [26].

14. Global Perspectives on Organoid Research

Advancements in organoid technology redefine research across geographies and disciplines. As multipotent stem-cell-derived cultured tissue surrogates, organoids capture physiology, functionality and cellular heterogeneity at the organ level. Organoids offer fast and efficient establishment from various human tissues, from healthy to malignant, enabling ex vivo evaluation of development, repair and disease [2].

International engagement in organoid research sustains momentum and spurs innovation, as governments support academic institutions and companies embark on exclusive programs. Yet global capacity remains uneven. A directorate-general at the European Commission stresses the critical importance of prevention, research and innovation, particularly in context of the COVID-19 crisis [13]. Countries typically cluster by shared interest: some focus on brain, inner ear or eye organoids, while others emphasize genito-urinary, gut, heart or lung systems.

More than 1,000 academic institutions worldwide investigate molecular and cell biology of organ development, regeneration and disease. Regulatory authorities also participate in initiatives on the reproducibility of human organoid models. The pharmaceutical industry applies organoids across numerous disease areas, from oncology to infection, immunology, neurodegenerative and inflammatory disease. Partnerships between academia and industry promote rapid translation of academic outputs into high-prestige grants, publications and pharmaceutical pipelines.

15. Conclusion

Recent advances in next-generation organoids have transformed human disease modelling and drug discovery. Organoids can be generated from diverse sources—including primary tissues, pluripotent and adult stem cells, cancer cells, and genetically engineered lines—and recreate aspects of human physiology and development previously only accessible through limited sample types such as embryonic tissues, human surgeries or biopsies, and transplanted animals. They have enabled rapid construction of personalized human disease models that are scalable to high-throughput drug screening and are applicable to a wide range of genetic disorders, infectious diseases, chronic illnesses, and cancer.

Recent innovations have significantly enhanced a number of key tools that are essential for structural and functional characterisation. These advancements include cutting-edge techniques such as multiplexed imaging and spatially resolved -omics, which have permitted their seamless integration into next-generation organoid culture systems. These state-of-the-art systems not only support quantitative measurements but also facilitate a deeper understanding of complex biological processes. Furthermore, new engineering solutions have dramatically increased the degree of control over the development of organoids. This has pushed forward the incorporation of previously missing physiological components. These components include essential features such as tissue interfaces, vascularisation, immune components, the establishment of body axes, and the integration of peripheral innervation, all while ensuring full electrophysiological functionality. Additionally, the rapid emergence of automation and high-fidelity quality control measures has elevated organoid systems to become robust platforms that are suitable for applications in chemical genetics, target validation, and large-scale drug screening in both academic research settings and the pharmaceutical industry. As a result, organoids are now routinely integrated with complementary disease modelling technologies, which enhances their utility and relevance in scientific research. Organoids serve as the foundational basis for bioengineered tissue transplants and thus hold the promise to drive a broad range of significant biomedical advances in the coming years and across multiple scientific disciplines. The ongoing developments in this field suggest a transformative impact on research methodologies and therapeutic strategies, potentially revolutionizing how we approach complex medical challenges in the future.

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