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Review

A Decade of Organoid Research: Progress and Challenges in the Field of Organoid Technology

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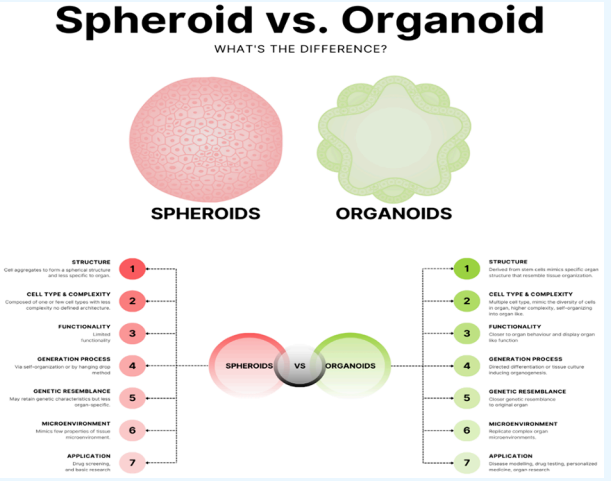
ABSTRACT: Organoid technology, revolutionizing biomedical research, offers a transformative approach to studying human developmental biology, disease pathology, and drug discovery. Originating from the pioneering work of Henry Van Peters Wilson in 1907 and evolving through subsequent breakthroughs, organoids are three-dimensional structures derived from stem cells or tissue explants that mimic the architecture and function of organs *in vitro*. With the ability to model various organs such as intestine, liver, brain, kidney, and more, organoids provide unprecedented insights into organ development, disease mechanisms, and drug responses. This review highlights the historical context, generation methods, applications, and challenges of organoid technology. Furthermore, it discusses recent advancements, including strategies to address hypoxia-induced cell death and enhance vascularization within organoids, aiming to refine their physiological relevance and unlock their full potential in personalized medicine and organ transplantation.



INTRODUCTION

To understand embryonic development, disease pathology, and drug discovery, scientists are using various immortalized cell lines and animal models and extrapolating the results to humans. However, recent "organoid" technology is helping scientists to understand developmental biology and disease pathophysiology in the human context and provides an excellent opportunity to draw conclusions about how drugs might interact in *in vivo*, thus potentially revolutionizing the field of drug discovery and personalized medicine. The term "organoid" refers to the cells growing in a three-dimensional structure with the property of self-organization and differentiation into different cell types of a particular organ under *in vitro* conditions. These organoids resemble the architecture of organs *in vivo* and are often called "miniorgans".[1,2] The term organoid was first reported in 1946 in a different manner; however, the meaning of organoid has evolved and refers to structures that are like organs with distinct features (Figure 2) and have been progressively used in *in vitro* biology. The earliest study of *in vitro* regeneration was reported in 1907 when Henry Van Peters Wilson reported the self-assembly of dissociated sponge cells into a whole organism.[3] Later, after Henry Van Peters Wilson, a few other groups reported this kind of phenomenon in amphibian pronephros[4] and chick embryos.[5] Decades later, in 1981, stem cell research began to bloom after the isolation of pluripotent stem cells (PSCs).[6] Later, the establishment of induced pluripotent stem cells (iPSCs) from human and mouse fibroblasts had a significant impact on stem cell and organoid research.[7] In 1987, Li et al. reported the formation of 3D ducts and lumen from breast epithelium and secrete milk proteins.[8] In 2009 in a groundbreaking discovery, Sato et al. reported the formation of 3D intestinal organoids in Matrigel using adult intestinal stem cells (cells expressing leucine-rich repeat-containing G protein-coupled receptor (Lgr5).[9] Thereafter, as illustrated in Figure 1, several other organoids have been generated mimicking different organs such as the liver, stomach, esophagus, breast, pancreas, kidney, retina, prostate, thyroid, lungs, testis, and brain.

The organoids can be generated either using adult stem cells (ASCs) or induced pluripotent stem cells (iPSCs).[10] The ASC-derived organoids are generated from stem cells present in adult tissue. The growth and differentiation of cells in organoids is supported by a media cocktail that provides essential signaling molecules required for the process. The

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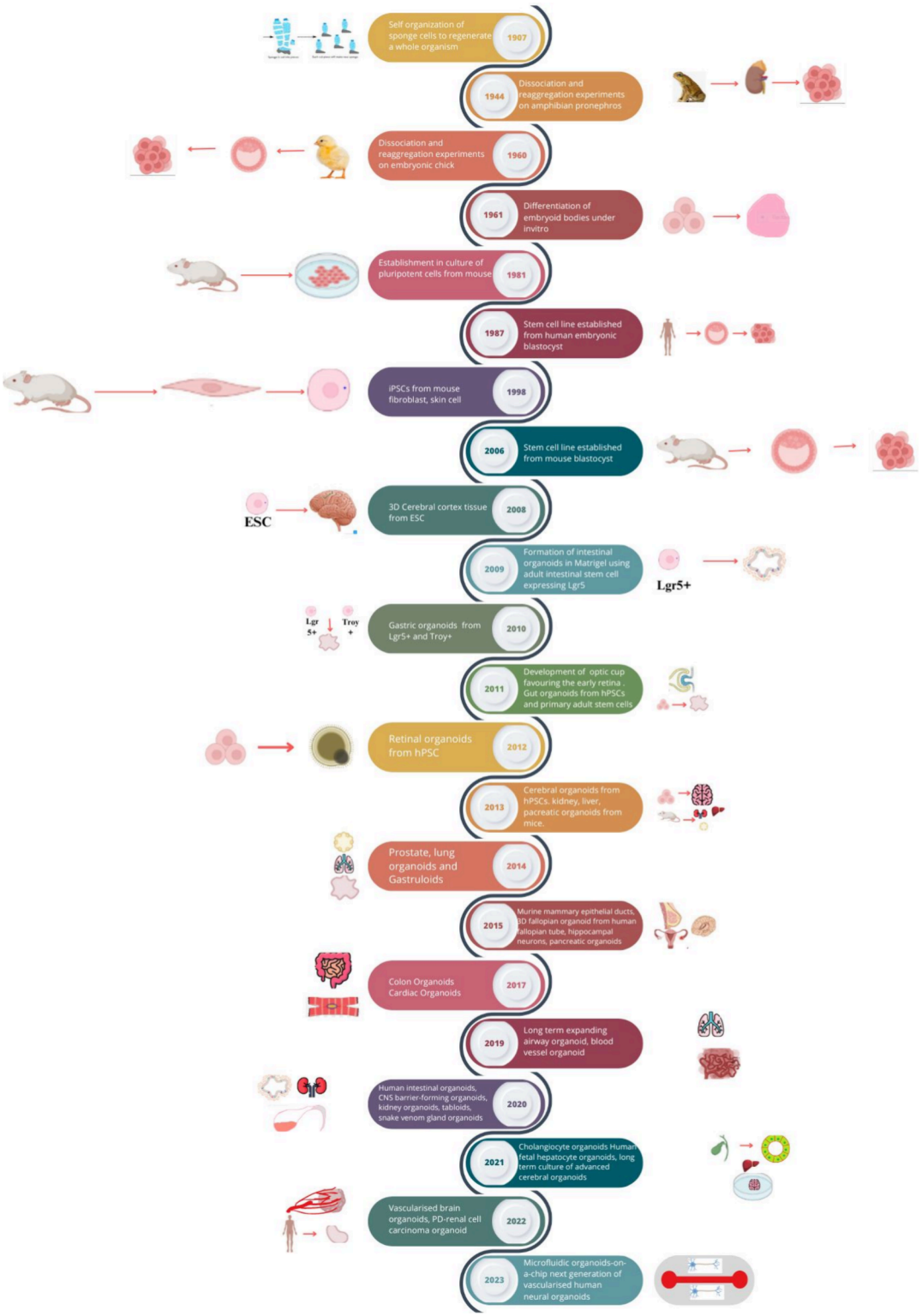

Figure 1. Road map of organoid development.

ASC organoids can also be made using patient-derived ASCs that help in disease modeling and precision medicine. The generation of embryonic stem cells (ESCs) or iPSC-derived organoids involves a multistep process of differentiation and provides hint-related development during gastrulation and organogenesis.[1] Human-induced pluripotent stem cells are obtained from fibroblasts and then reprogrammed to resemble embryonic-like states. iPSCs or iPSC-derived organoids are widely used by the research community due to reproducibility, differentiation potential, and avoidance of ethical concerns associated with human ESCs. Additionally, iPSC-derived organoids have been an asset to establish organoids from organs with limited self-renewal capabilities such as the brain, heart, and kidney. ESCs are present only in early embryos and are totipotent in nature. ESC-derived organoids are very effective models for investigating the development of genetic

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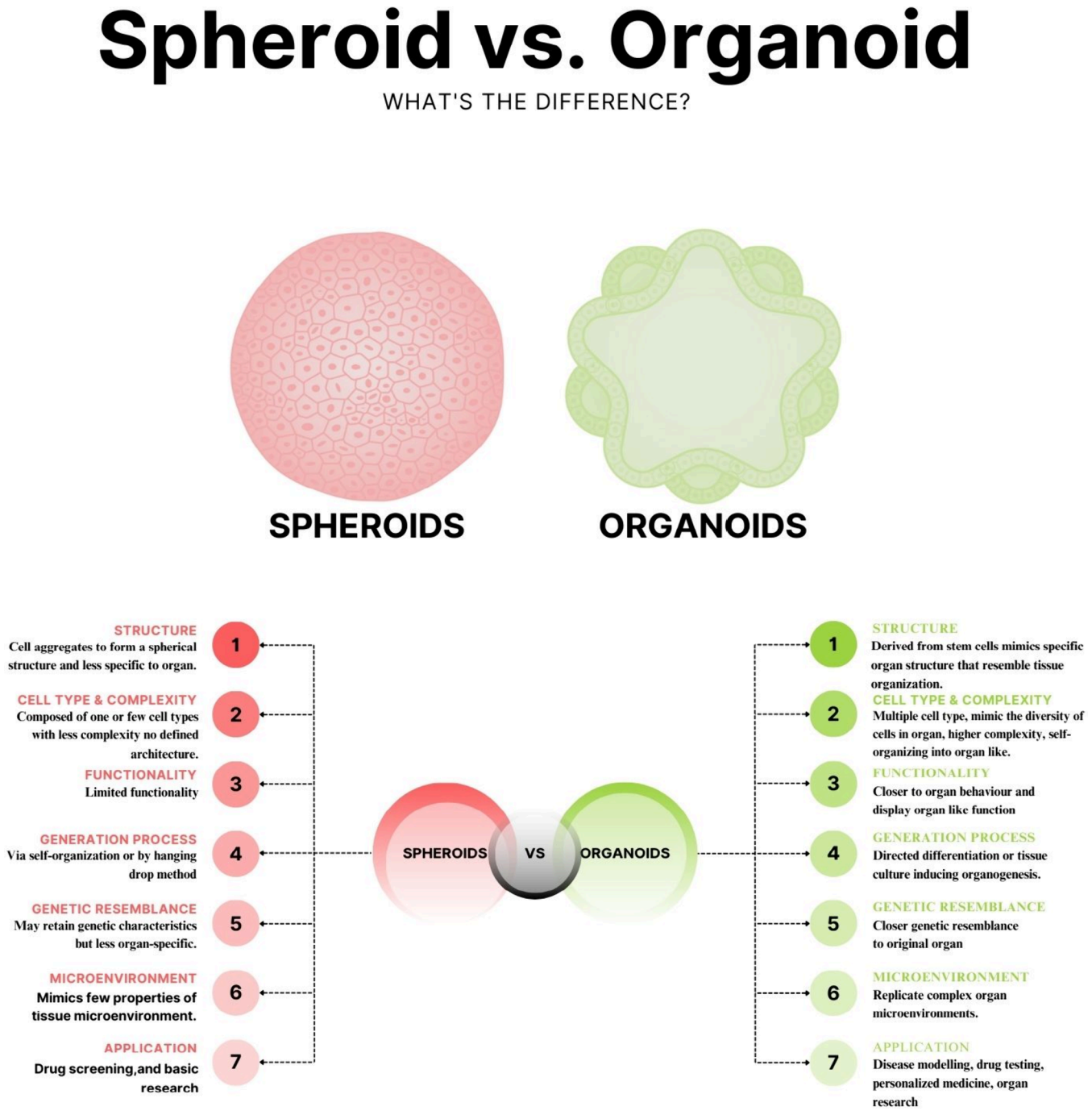

Figure 2. Characteristics of spheroids vs organoids.

disorders and infectious diseases, especially in organs with very limited regeneration power (brain). These organoids are more complex (enabling organoids from all three germ layers) and might include mesenchymal cells, epithelial cells, and sometimes endothelial cells. In ASC-derived organoids, only the epithelial parts of the organ are present, while nerves, vasculature, etc. are absent. Additionally, the generation of organoids from ESCs/iPSCs is more time-consuming and complex compared to ASC-derived organoids.

Tongue Organoids. In 2013, Hisha et al. developed tongue organoids from BMI-1-positive stem cells that can be engrafted in mice. Additionally, the organoids developed from the mice treated with carcinogens had abnormal morphology.[11] Later, in 2015, Aihara et al. developed taste bud organoids from Lgr5+ cells in the circumvallate papillae.[12] More recently it has been shown Sox9 and FoxC1 resulted in the differentiation of ESCs into the salivary glands. This engraftment in mice showed the morphology and function (saliva secretion) of salivary glands.[13]

Gastric Organoids. Clevers's group, after the successful generation of intestinal organoids, also developed gastric organoids from the Lgr5+ and Troy+ cells in the pyloric gland and corpus gland, respectively, in mice.[14] A similar methodology was also used for human adult gastric organoids for a durable period. Later, McCracken et al. in 2014 developed gastric organoids from human PSCs by the

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manipulation of WNT, BMP, FGF, retinoic acid, and EGF signaling pathways and culture in a 3D environment. These organoids have predominantly pyloric lineage.[15]

Intestinal Organoids. After the development of intestinal organoids in 2009 by Sato et al., the long-term culture for adenocarcinoma and human colon was developed by the same research group in 2011.[16] In the same year, Spence et al. directed the differentiation of human PSCs into intestinal organoids. These organoids when *in vivo* transplanted in mice developed epithelial features (presence of enterocytes, goblet cells, Paneth cells, tuft cells, enteroendocrine cells, and brush border enzymes) and a smooth muscle layer. The transplanted tissue showed intestinal functions and was supported by mouse vasculature.[17]

Liver Organoids. The liver has very good regeneration power. Despite this, the *in vitro* expansion of hepatocytes is very poor. The Clevers group noted in 2013 that, in the healthy adult liver of mice (*Lgr5-LacZ knock-in*), Lgr5-LacZ is not expressed. However, damage to the liver with CCl4 resulted in a small number of Lgr5+ cells reported near the bile duct. These Lgr5+ cells were expanded as 3D organoids in Rspo-1 (Wnt agonist) containing medium and can be differentiated *in vitro*. These organoids can also generate functional hepatocytes when transplanted in FAH−/− mice.[18] Takebe et al. developed the method for long-term culture of human liver organoid culture. The human-iPSC-derived liver buds were also used to generate vascularized and functional liver after transplantation.[19] Later, in 2018, the Clevers group along with the Nusse group established the long-term culture of human and mouse hepatocytes as 3D organoids with engraftment efficiencies.[20]

Pancreatic Organoids. The development of pancreatic organoids has been reported with plating of mouse embryonic pancreatic progenitor in the 3D matrix (Matrigel).[21] Organoids from the adult pancreas have also been generated with the possibility of differentiating into a ductal and endocrine lineage with transplantation.[22]

Brain Organoids. The studies prior to the work of Watanabe and colleagues were mostly dealing with mostly 2D or very simple aggregates that lacked the complexity of the brain. Watanabe and colleagues developed the 3D culture of the brain using human and mouse PSCs.[23] In 2013, Lancaster et al. used an improved method earlier developed by Watanabe and colleagues by growing embryoid bodies (EBs) in Matrigel. These organoids when transferred to a bioreactor develop different regions of the brain including the retina, dorsal cortex, ventral forebrain, midbrain−hindbrain boundary, choroid plexus, and hippocampus.[24] Later organoids were generated that specifically modeled the different regions such as the cerebellar, hippocampal,[25] and midbrain.[26] In 2016, a miniature spinning bioreactor was developed that generates forebrain-specific organoids from human iPSCs. The developed organoid showed the features of human cortical development, neurogenesis, gene expression, and human-specific outer radial glia cell layer. With the use of this technology, midbrain and hypothalamic organoids were also generated.[27]

Retinal Organoids. The primary reaggregation experiments with chick retina exhibited the property of self-organization *in vitro* conditions forming rosettes.[28] However, the dissociated cells form correctly laminated structures when cultured in the presence of Wnt2b.[29] Later the culture of mouse embryonic stem cells derived in 3D resulted in the development of retinal primordium organoids favoring the early retina. These optic cup organoids were also developed from human ESC culture, much larger than the mouse organoids developed from mouse ESCs. These human-ESC-derived organoids developed into multilayered tissue containing both rods and cone cells, while this differentiation was very rare in mouse-ESC-derived organoids. The process of photoreceptor accumulation in human ESCs can be accelerated with Notch inhibition.[30]

Kidney Organoids. The reaggregation experiments with chick embryonic kidneys showed the ability of self-organization under *in vitro* conditions.[5] Ureteric bud organoids with mesodermal properties were developed from human PSCs when grown in the presence of fibroblast growth factor (FGF-2) and bone morphogenetic protein. When these organoids were later provided with 4 days of exposure to retinoic acid (RA), activin A, and BMP-2, they developed renal progenitor markers. Further maturation was done using 3D culture where the human cells were cocultured in the presence of mouse embryonic kidney cells and developed chimeric ureteric buds.[31] Subsequently, the metanephric mesenchyme (MM) was derived from pluripotent stem cells (PSCs) on human and mouse when cultured in the presence of activin, BMP-4, and Wnt activator CHIR99021 followed by RA and FGF-9.[32] These metanephric nephron progenitor cells generate very complex 3D kidney structures under *in vitro* conditions.[33] The organoids have glomeruli with podocytes and renal tubules with proximal and distal regions and clear lumina. Upon transplantation, the glomeruli are efficiently vascularized.[33] In 2015 Takasato et al. developed kidney organoids using human iPSCs that contain multiple lineages and model human nephrogenesis. Inside the organoids, the nephron is segmented into proximal and distal tubules, loops of Henle, and glomeruli. The transcription profile of these organoids closely resembles that of the human first-trimester kidney.[34] Additionally, these organoids showed functionality to dextran and nephrotoxic cisplatin.[2] Recently, long-term cultures of kidney organoids (tubuloids) that can be cultured for 20 passages were established from the human and mouse kidneys. The kidney tubuloids were also developed from the urine of subjects with cystic fibrosis (CF). The organ-on-a-chip using these tubuloids reported transepithelial transport function.[35] Additionally, single cell RNA sequencing has delineated differences between two major protocols for degenerating kidney organoids by identifying at least 12 distinct kidney cell types in both protocols. It also revealed the presence of off-target nonrenal cell types at similar ratios in organoids derived from human induced pluripotent stem cells and human embryonic stem cells.[36]

Lung Organoids. The lung cancer cells were used in gas−liquid interface culture conditions, where they reorganized and differentiated into organoid structures with histological structures resembling the original tissue.[37] In 2009, basal cells were reported as stem cells in the lungs of humans and mice with integrin alpha 6 and nerve growth factor receptors as surface markers. Later, in 2013, alveolar epithelial type 2 (AT2) cells were cocultured with lung fibroblasts with PDGFRα+ (platelet-derived growth factor receptor alpha+) readily forming alveolospheres. The group also established AT2 cells as stem cells in adult lungs.[38] In 2014, alveolar epithelial spheroids were generated using human alveolar epithelial progenitor cells (AEPs).[39] The use of human pluripotent stem cells was also used to generate human lung

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organoids with structural features resembling those of native lungs.[40] Similarly, human airway organoids were established from the bronchoalveolar resections with basal cells, functional ciliated cells, mucus-producing cells, and CC10-secreting club cells.[41] Currently, the human proximal airway and distal alveolar organoids can be derived successfully.

Other Organoids. Besides the above-mentioned organs, organoids can be generated from various parts of the body. Some of the organoids that are not widely studied but are developed are described in this section.

Mammary Organoids. Recently, Jamieson et al. developed mammary organoids from the basal mammary epithelial cells (MECs) that resemble the features of mammary tissue architecture and function. These complex organoids have polarized luminal cells on the inner compartment and have a milk-producing capacity, while myoepithelial cells are located on the outer side. The cultured cells have their regenerative capacity intact as they produce ductal outgrowth *in vivo*.[42]

Prostate Organoids. Prostate organoids can be generated from mouse or human prostate epithelia and develop both luminal and basal cells. Organoids generated from either the CARNS (castration-resistant Nkx3.1-expressing cells) or normal prostate epithelia can be expanded for the long term with both luminal and basal cells, and these organoids show active androgen receptor signaling.[43] Similarly, Clevers's group also developed a system that supports the long-term culture of human prostate organoids containing both basal and luminal cells. Both the basal and luminal cells can give rise to prostate organoids, but the organoids derived from the luminal cell are closer to prostate glands.[44]

In 2012, Antonica et al. showed that mouse ESCs differentiate into thyroid follicular cells with transient upregulation of NKX2-1 and PAX8 and that these thyroid follicular cells organize in 3D follicular architecture when treated with thyroid stimulating hormone, thyrotropin. When these organoids were transplanted into thyroid mice, these thyroid follicles initiated the symptomatic recovery and recovered thyroid hormone levels.[45] Cardiovascular organoids were developed by a very simple and efficient method that allows the formation of cardiac-muscle-like tissue in EBs, and contracting cardiovascular organoids were formed just by manipulating the stiffness of the material used for cell adherence.[46]

APPLICATIONS OF ORGANOIDS

Organoids mimic the 3D architecture of organs, recapitulate the organ biology, and offer a simple and easily available cellular model. Organoid technology holds strong promise in the medical field that may fulfill the unmet needs in the field with a wide range of translational applications. Some applications of organoid technology are described in this section.

Genetic Disease Modeling. The beauty of organoid technology is the modeling of genetic disease and thus development of potential new therapeutic strategies. The first and successful organoids were developed by Hans Clevers from adult intestinal stem cells. Later this organoid technology was used for the study of cystic fibrosis (CF), caused by a mutation in the CF transmembrane conductance regulator (CFTR).[47] Later a quick assay for CFTR function was developed that can help in a quick diagnosis and in drug screening especially personalized medicine and functional research.[48] Similarly the kidney organoids developed by Menendez et al. from patients with polycystic kidney disease paved the foundation for research in genetic disorders related to kidney.[49] Eventually the technology was used for functional research for Fabry disease.[50] Likewise, retinal organoids have been used for associated retinal genetic disorders.[51,52] Hypoplastic left heart syndrome, which is the most common congenital defect in human, has also been modeled using organoid technology with NKX2-5 and HAND1-knockout cardiac organoids.[53] Similarly, brain organoids have been used for neurodevelopmental genetic disorders such as microcephaly[24] and Down syndrome.[54]

Nongenetic Disease Modeling. Recently there has been an increasing necessity of incorporating nongenetic factor scores in studying disease models. Organoid models provide an excellent controlled environment for understanding nongenetic factors like hormonal effects, epigenetics, microbial infection, and immunological influence. Owing to the advancements in stem cell technology, kidney organoids derived from human pluripotent stem cells have emerged as powerful models in mimicking key aspects of kidney structure and function, which make them valuable tools for research. A study examined the differentiation and maturation processes of hPSC-derived kidney organoids, which were generated to understand human kidney development and acute kidney injury (AKI). These organoids efficiently induced both nephron progenitor cells (NPCs) and stromal progenitor cells (SPCs), resembling the human embryonic kidney. Immunostaining and flow cytometric analyses confirmed the presence of key markers and the maturation of kidney structures over time. The organoids' response to cisplatin-induced injury was also investigated, highlighting selective damage to proximal tubular cells and subsequent intrinsic repair mechanisms. Prolonged cisplatin exposure led to incomplete repair, characterized by tubular atrophy and interstitial fibrosis. Further, single nuclear RNA sequencing (snRNA-seq) revealed distinct transcriptional changes during the transition from intrinsic to incomplete repair, providing insights into the cellular and molecular dynamics of kidney injury and repair. These findings underscore the utility of kidney organoids as a model system for studying organ development, injury, and repair mechanisms.[55]

Drug Discovery. Current drug discovery has many limitations such as unpredictable outcomes, toxicity, and cumbersomeness. The organoids developed from the patients of cancer, infectious diseases, or developmental diseases show clinical resemblances and thus could be beneficial for drug discovery. Recently the ERK inhibitor for treatment of human primary cancer organoids has been identified[56] along with two compounds that are found beneficial for colon cancer associated with APC mutation.[57] Similarly, virus infected brain organoids have also been used for antiviral drug discovery and validation of antiviral drug candidates such as duramycin, ivermectin, and azithromycin.[23,58] High-throughput drug screening have been used in drug discovery using only intestinal organoids.[59] Kidney organoids derived from human pluripotent stem cells allow for the assessment of nephrotoxicity and therapeutic effects in a 3D environment that closely mimics human kidneys. Fully automated 3D imaging and machine-learning approaches enable detailed profiling of nephron segment-specific responses to drugs. This advanced technology helps identify nephrotoxic or therapeutic compounds, as demonstrated with cisplatin-induced kidney injury models. The use of kidney organoids in high-throughput

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Table 1. Commercially Available Organoids

organ name	type	disease model or developmental model	company	ref
kidney	organoid	disease model: polycystic kidney disease, kidney injury	STEMCELL Technologies	Takasato et al.[34]
prostate	organoid	disease model: Prostate cancer, benign prostatic hyperplasia (BPH)	3D Biomatrix	Goa et al.63
heart	organoid	developmental model: cardiogenesis, heart diseases	Ncardia	Mills et al.64
retina	organoid	disease model: retinitis pigmentosa, age-related macular degeneration	ORGANOID Technology	Zhong et al.65
liver	spheroid	disease model: hepatitis, liver fibrosis	Biomatrices	Bhise et al.66
lung	organoid	disease model: lung cancer, pulmonary diseases	Pandorum Technologies	Dye et al.40
intestinal	organoid	disease model: inflammatory bowel disease (IBD)	Thermo Fisher Scientific	Sato et al.[16]
brain	organoid	disease model: Alzheimer's disease, brain cancer	Sigma-Aldrich	Lancaster et al.[24]
pancreatic	organoid	disease model: pancreatitis, pancreatic cancer	Merck	Boj et al.67
kidney	organoid	disease model: renal fibrosis, nephrotoxicity	ATCC	Takasato et al.[34]
mammary	organoid	disease model: breast cancer, lactation	Corning	Sachs et al.68
colon	organoid	disease model: colorectal cancer, colitis	STEMCELL Technologies	Sato et al.[9]

screening can potentially streamline drug discovery and improve the assessment of drug safety and efficacy.[60]

Toxicity Assessment. One of the main reasons for the failure of drugs during clinical trials is the associated toxicity that generally is discovered in late clinical studies or even at the launch stage. In this condition, organoids provide a better opportunity for toxicity assessment, since they mimic the physiological tissue reactions. Therefore, organoid technology could be the future of toxicity studies. Nowadays several companies offer platforms having heart, liver, and lung organoid systems with single recirculating perfusion along with various individual organoids commercially available for drug discovery and toxicity assessments (Table 1). These kinds of platforms could be used to assess the toxicity of drugs in whole organs.[61] The kidney is highly susceptible to drugs and toxicants due to its significant blood flow and active drug transport in renal tubular epithelia. Despite comprising only 0.4% of the total body weight, the kidneys receive 25% of the cardiac output, leading to high drug exposure. Additionally, renal water reabsorption concentrates the glomerular filtrate in tubular lumens, resulting in elevated drug concentrations. Kidney organoid models have proven valuable for drug toxicity assessment as they accurately replicate the complex cellular architecture and functionality of human kidneys.[62] Overall, organoids with great resemblances to the human genetic makeup not only serve as the best alternatives of animal models but also, if derived from patients, can be the best fit to offer precision medicine.

Personalized Medicine in Cancer Treatment. The traditional 2D culture methodology does not provide the actual model of a tumor and thus results in failures of drug discovery in clinical trials.[69,70] However, the use of patient-derived organoids provides an advantage over traditional drug discovery because it recapitulates the patient tumor type and thus could be used to identify the potential therapeutic strategy in a patient-specific or personalized manner. Various organoid biobanks have been generated that can enhance drug discovery. One of the best examples of organoid technology is CF treatment, where clinical applications of organoid technology have been established in personalized manner.[71] Organoids from rectal cancer have also been used to assess the impact of radiotherapy[72] and chemotherapy.[73] Additionally, the organoid biobank for pancreatic ductal adenocarcinoma (PDAC) has also been developed for drug discovery, and compounds identified in this strategy are under clinical trial.[74] Collectively, the organoid biobank from a patient could be a powerful tool for disease modeling and drug discovery in a patient-specific manner.

Organ Transplantation. Another major advantage of organoid technology is organ transplantation in a patient with organ failure. Researchers have made sincere efforts in this direction and have been successful with some limitations. The transplantation of an intestinal organoid from human PSCs in mouse showed successful expansion and maturation.[75] Similarly, in rats with retinal disorders, retinal organoids form synaptic connections and projections in the host rat.[76] The transplantation of cerebral organoids in a rat stroke model reduced brain infarct volume, and neurological motor function improvement was observed.[77] Much other research has been performed that forms the theoretical basis of organ transplantation using organoids. However, the organoids are less mature in comparison to natural organs because of either heterotypic cell population or functional immaturity. Despite these facts the use of organoids still can be meaningful as an alternative strategy to real organ transplant.[78] However, extensive research is still required to put this strategy in clinics.

Hurdles in Organoid Technology. Organoid technology holds great and attractive opportunities for various applications and offers an innovative perspective toward biomedical and clinical translational research. However, there are many speed bumps that need to be addressed for future research.

One the major components in organoid technology is the use of a matrix that helps in organoid development. A basic matrix such as Matrigel and basement membrane extracts have component variability from batch to batch.[79] This kind of batch variability affects the reproducibility of results, which is one of the critical factors in biological science/research. Additionally, they could have an impact on the immune response system.[80,81] The current organoid technology also fails to model multiorgan pathologies. Some recent research such as the use of a coculture approach (such as hPSC intestinal organoids and neural crest cells)[82] and organ-on-a-chip technology (that enables association between preformed organs) has provided some leads to solve this problem.[83] Another important factor that is hampering the growth of organoid technology is tissue maturation. The diffusion of nutrients and oxygenation are two components that play important roles in tissue maturation.[84] When organoids are grown for longer periods, they start showing apoptosis or necrosis due to anoxia.[85−87] To overcome this problem, a new strategy needs to be devised for the perfusion of organoids. Some research has been done in this field where brain organoids were re-embedded in Matrigel with endothelial cells

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to vascularize the organoids.[88] Recently, a study has reported the transplantation of organoids in animals resulting in vascularization[89] from the host supporting the transportation of oxygen and nutrients.[90]

In a nutshell, organoid technologies have been used in various applications in the preclinical stage. However, the research is still in its infancy period and future extensive research could strengthen its application especially in clinics.

CONCLUSION

As organoids expand in size, their innermost cells face reduced oxygen availability, leading to a hypoxic microenvironment and subsequent cell demise. To address this challenge, innovative strategies emerge. The introduction of artificial blood substitutes, such as perfluorocarbons (PFCs) or hemoglobin-based oxygen carriers (HBOCs), presents a promising avenue to circumvent hypoxia-induced cell death within organoids. Simultaneously, encouraging vascularization stands out as a complementary approach. Coculturing organoids with endothelial cells or utilizing animal models for organoid transplantation triggers the development of intricate vascular networks, providing a lifeline for core cells by facilitating sufficient oxygen and nutrient supplies.

Refining these integrated strategies holds promise, yet ongoing improvements are crucial to fully unlock the potential of organoids, accurately replicating diverse physiological conditions.

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Notes

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