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Organoids: inception and utilization of 3D organ models

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ABSTRACT: Over the previous decade, one of the most exciting advancements in stem cell technology has been the development of organoid culture system. Organoids are new research tools created in-vitro, to form self-organizing 3-Dimensional structures that encompass some of the crucial characteristics of the represented organ. Organoids are grown from stem cells from an organ of interest. There are potentially as many types of organoids as there are different tissues and organs in a body. It is challenging for scientists to understand the underlying mechanism of biological processes with complex spatial cellular organization and tissue dynamics. Also, how they are disrupted in a disease is impossible to study in-vivo, but discovery of organoids is revolutionizing the fields of biology. Since success in these platforms will be restricted without the proficiency to alter the genomic content, genome engineering was also applied in recently discovered organoid cultures for correcting mutations. This review discusses the history, culturing methods, current achievements, and potential applications of this technique. These applications involve drug screening, personalized oncological medication, disease modeling, regenerative medicine, and developmental biology. The study of organoids has provided a novel platform in biological sciences, with new approaches for stem cell technology.

Keywords: Organoids; Adult stem cells; Pluripotent stem cells; Genome engineering; Bioprinting.

1. INTRODUCTION

Culturing miniature organ in a dish sounds like science fiction. But with all the advancement in science and thriving stem cell technology and bioengineering, this is no longer a fantasy. Scientists are now able to grow artificially mass of cells into a three-dimensional (3D) structure known as organoids [1].

The concept of 3D cell culture has been around for over a century when H.V. Wilson discovered that sponge cells rearrange and sort out, even after mechanical separation, and grow into functional organisms. Organoids represent cell-derived tissue and organ-like structures composed of one or more cell types, formed by 3D cell culture creating mini, simplified organs that retain some physiological functions [2].

They are grown in a three-dimensional environment from one or a few cells, from a tissue, embryonic stem cells, or induced pluripotent stem cells or progenitor cells from an organ of interest. 3D cell culture has grown and become increasingly widespread since it can now be applied to mammalian cells. It is only because of the recent advent of the field of stem cell biology, the potential of stem cells to form organs in vitro was

realized. Due to this 3D cell culture techniques have become widely applicable. Even genome engineering is also applied to organoids that can be used to induce certain changes in a different genetic background to overcome certain limitations [3].

Organoids are wide in characteristics. They can mimic the recapitulated structures of tissues and organs. They have the potential to be used in the patient-specific treatment, avoiding immune rejection, and overcoming many ethical issues. Organoid culture is an advanced tool with tremendous potential to influence life sciences, as currently used animal models and 2-dimensional (2D) organ culture are not like humans. Thus, organoids can outperform current in vitro systems and replace a significant portion of animal-based toxicology studies. They can also help understand how tissues take up and react to pharmaceuticals. Scientists are aiming to expand organoids utilization in future [1, 2].

2. CULTURE METHODOLOGY

Organoids are new research tools derived from human pluripotent or adult stem cells or somatic cells in vitro, embedded in Matrigel or Extracellular Matrices (ECM) to form self-organizing 3D structures. These structures simulate many of the functions of needed organs. For example intestinal, endometrial, hepatic, renal and other such organoids are derived from adult stem or progenitor cells. Whereas, cortical brain organoids among other organoids have been created from pluripotent stem cells (PSCs).

2.1. General method

Cells or tissues to culture organoids are derived from patient's induced pluripotent stem cells (iPSCs) through tissue biopsy. They are then differentiated, and the cells are sorted out. The spatially restricted lineage commitment occurs then forms the organoids which further has many applications to study on. Successful organoid formation also requires the careful orchestration of spatio-temporal cues from growth factors and supportive matrices to simulate the need of each organ type [4].

There are few methods to culture intestinal organoids; one such method is the general submerged method, where centrifuged cells are suspended in ECM are accompanied by organ-specific growth and patterning factors. The culture medium is then washed with cold (277.15K) phosphate buffered saline and allowed to harvest adding cold organoid harvesting solution. The ECM then polymerize leaving intact organoids. Therefore, the resulting organoids are then either used immediately or stored in biobanks [4].

In general, liver organoids are cultured by developing primary human bile duct cells in vitro, using ECM, into a three-dimensional structure. Brain and Retina organoids are extracted from stem cell differentiation. They are further treated and formed in low adhesion movement through embryoid bodies. Pancreas organoid culture are grown in ECM by isolating the duct cells from the human pancreas [2].

2.2. Three-dimensional (3D) bioprinting

The 3D bioprinting method involves layer-by-layer printing of biomaterial, using bio-ink, which is laden to the printer. Hydrogels, such as Matrigel matrix and collagen, are usually used as a source for bio-inks as they ensure embedding along with positioning of the printed biomaterial [5]. Recently, laser direct write (LDW) technologies are being used in place of bio-inks (Dr. Doug Chrisey, Prof., Tulane University), as they provide single-cell spatial resolution [6]. Some commonly used methods under this technique involve, laser-assisted printing, inkjet, micro extrusion-based printing, tissue fragment, microvalves, and scaffold-free spheroid-based bioprinting (Kenzan method) [7]. The use of microfluidic systems has also been in practice lately (Dr. Noah Malmstadt, Ass. Prof., University of Southern California) [6].

Although this method of generation aids in the creation of targeted and personalized therapeutics, hardly any structures are efficient enough for concoction of whole organs capable of transplantation [8]. The first ever transplant of bioprinted organoids was of the urethra and bladder, which was made possible by The Wake Forest Institute of Regenerative Medicine [7]. Recently, in Korea a respiratory epithelium model has also been created to study the viruses that may infect the respiratory tract, in the wake of the ongoing pandemic [8]. The coalescence of genome editing, via clusters of regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), and organoid technologies aid in evaluation of DNA repair of targeted and patient-specific mutations, as well as modeling several heritable and inheritable diseases [5].

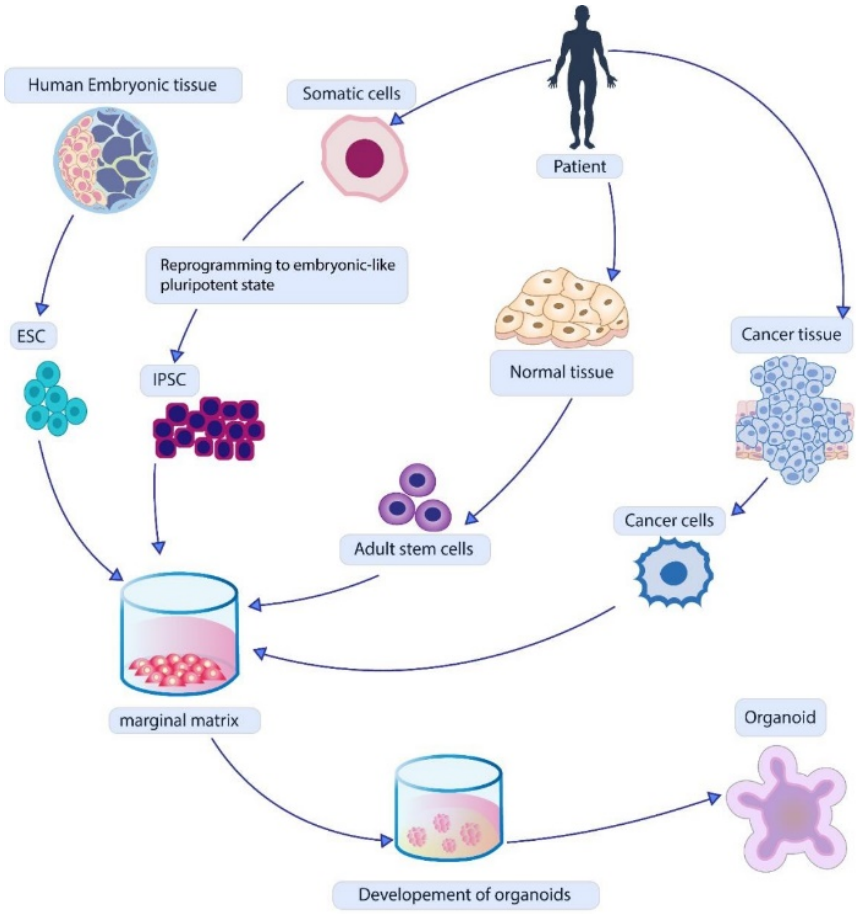

Figure 1. Schematic representation of culture methodology for organoids.

3. CLASSIFICATION OF ORGANOIDS

Organoids can be fundamentally characterized into those derived from PSC, i.e., either induced or embryonic, and adult-stem cell (ASC) or progenitor cell. The ASC-derived organoids are more accurately modelled and aid better in congenital and non-congenital disease modeling along with personalized medicine [9]. This is due to the shorter generation time of ASC-derived organoids. These organoids are composed of previously tissue-specific progenitor cells. These tissues can be stored for future use by cryopreserving them.

The precursor cells for PSC-derived organoids are iPSC or PSC cell lines, which are easier to access than ASC-derived cells. These cells possess the ability to undergo differentiation into the three germ layers,

ectoderm, mesoderm, and endoderm, when induced by growth factors [10]. Their capacity to differentiate into any cell type and proliferate indefinitely is valuable for creating multiple lineages. The organoids formed are previously prompted with a specific genetic code. It also provides as the only means for generation of organoids with neural tissue.

The common component used in the culture medium of both types of organoids is the ECM or Matrigel. The culture medium may be specific to the signaling environment and tissue of the organoid, which cause cell differentiation [9].

3.1. Skin organoid (with hair follicle) – Ectoderm-derived

Skin is an organ that plays its role in the recognition of external stimulus, homeostasis, and retaining body fluids. The latest research by Dr. Jiyoon Lee, states the generation of complex skin from human pluripotent stem cell (hPSC), with the incubation period of approximately 150 days. A cyst-like skin organoid is then seen which has roots extending radially outward. It is composed of a complex structure including epidermis, dermis, and hair follicle with sebaceous glands. The hair follicle starts appearing by about day 70. Besides, there are sensory neurons and Schwann cells that target the Merkel cells that are present. This structure is identical to the fetal facial skin in the 2nd trimester. This study is beneficial in providing insight into the skin and facial reconstruction. Applications may include disease modeling and reconstructive surgeries [11].

3.2. Lung organoid – Endoderm-derived

Upper-airway lung organoids can be cultured using single basal cells that self-organize. It has also been identified that bronchioalveolar stem cells co-cultured with lung endothelial cells, or Alveolar Type 2 (AT2) cells co-cultured with lung fibroblast showed the potential for differentiation. In recent studies, Rawlins' lab has generated the first human self-renewing lung organoids derived from embryonic lung and Clevers' lab created organoids with ciliated, club, goblet and basal cells. These models suggest the maintenance of regenerative activity [10]. Air-liquid interface (ALI) culturing is till date considered the most efficient and well-characterized model. These models prove their utilization in fields like toxicology and drug-screening due to the deposition of aerosol droplets on damp cell surfaces, which resembles the deposition of powders on the surface of the airways. The before mentioned organoid cultures may be useful for the study of processes involving homeostatic regulation of lung tissue and factors affecting lineage-specification of stem cells [12].

3.3. Cerebral organoid – Ectoderm-derived

3D models initially comprised of neurospheres, neural aggregates, neural rosettes, cortical spheroids, and finally whole-brain organoids. Organoid cultures containing Matrigel and specific levels of growth and signaling factors (Bone Morphogenetic proteins (BMPs), Wingless-related integration site (Wnt), Sonic hedgehog (Shh), Fibroblast growth factor (FGF)) cause enlargement and differentiation of neuroepithelium [13] leading to development of neural tube-like buds. Instead of utilizing a spinning bioreactor [10], miniature spinning bioreactor (SpinΩ) can be used, or even an Extracellular scaffolding material may allow extension and self-organization of neural buds into specific regions of the brain. The latest research on fused or cocultured organoids unveils the migration of interneurons from ventral telencephalon to the dorsal region as in the human brain [13]. Some experiments have even given surprising results like neuron maturation leading to a partial response to stimuli (light) due to the presence of retinal components in the organoid. Single cell

RNA sequencing reveals the cortical cells of the organoid mimicking gene expression similar to that of the human fetal neocortex [14].

3.4. Hepatic organoid – Endoderm-derived

Unlike the organoid, the organ is composed of mesoderm-derived hepatic cells [10]. These organoids can be generated by iPSC, Embryonic stem cell (ESC), hepatoblasts and adult tissue-derived cells. First morphologically similar structures were developed in 2001 but were short-lived. First embryonic liver bud organoids that matured and were functional in mice comprised of a combination of PSC-derived hepatocytes, Mesenchymal stem cells (MSCs) and Human Umbilical vein endothelial cells (HUVECs). Later iPSCs differentiated into endoderm, endothelium, septum transversum and were able to form cholangiocyte organoids too [10]. Adult tissue-derived organoids are clonogenic due to Leucine-rich repeat-containing G-protein-coupled receptor 5 positive cells (Lgr5+). Lgr5 hepatoblasts can form hepatocytes and cholangiocyte (bipotent) [15, 16]. In a recent study, Vallier lab modified the culture environment described by Huch et al. and generated extrahepatic biliary organoids. These further lead to the formation of tube-like structures similar to bile duct (involved rat, cat, and dog models also), which altered the gallbladder wall in mice. Nusse and Clevers labs have worked upon the generation of organoids from mouse ASCs. Human adult hepatocytes are yet to be created [10]. Table 1 portrays the various types of organoids that have been created till date.

Table 1. Different types of organoids with their classification, patterning factors and days taken for differentiation.

Type of organoid	Derived from		Patterning factors	Days for differentiation	References
	Stem cell	Germ layer			
Skin (Hair)	PSC	Ectoderm	Transforming growth factor β (TGFβ) inhibitor, BMP, FGF-2	~ 120	[10, 11]
Lung	ASC and PSC	Endoderm	TGFβ, FGF, Wnt	~ 50	[10, 17]
Kidney	ASC and PSC	Mesoderm	CHIR99021 for activating Wnt and FGF-9	21-35	[10, 18]
Brain	PSC	Ectoderm	Dual SMAD and Wnt inhibition	60	[10, 19]
Intestine	ASC and PSC	Endoderm	EGF, Wnt agonist R-spondin (RSPO), Noggin	~ 10	[10, 20]
Liver	ASC and PSC	Endoderm	Hepatocyte growth factor, EGF, FGF, RSPO-1, cyclic AMP, TGFβ inhibitor	15	[10, 21]
Pancreas	ASC and PSC	Endoderm	EGF, FGF, RSPO-1, Noggin	7	[10, 22]
Thyroid	PSC	Endoderm	BMP, FGF	5-21	[10, 23]
Fallopian tube	ASC and PSC	Mesoderm	TGFβ signaling	21	[10, 24]
Endometrium	ASC and PSC	Mesoderm	RSPO, EGF, FGF-10	~ 7-14	[10, 25]
Mammary gland	PSC	Ectoderm	EGF, Forskolin, insulin, hydrocortisone	~ 10	[10, 26]
Retina	PSC	Ectoderm	Wnt inhibition	~ 30	[10, 27]
Inner ear	PSC	Ectoderm	BMP4, TGFβ, Wnt activation	16-20	[10, 28]
Cardiac	PSC	Mesoderm	Wnt/β-catenin signaling via CHIR9902, BMP- 4, Activin A	15-20	[10, 29]

4. APPLICATIONS

4.1. Disease modeling

Organoids, considered as in-vitro models of living organisms especially humans, find their primary application in modeling of a variety of diseases. Besides being analogous in structure and shape to the actual organs, they are efficient in performing their tasks as well. Following are a few diseases, describing the efficacy of these mini organs.

4.1.1. Digestive disease

Digestive diseases refer to the disorders of the digestive tract which is also called to be as the gastrointestinal (GI) tract. Certain conditions may range from mild to serious. It can also lead to cancer and other such severe conditions. A common bacterium, *Helicobacter pylori* that causes fatal gastric diseases including gastric cancer and peptic ulcer disease, is treated with gastric organoids to study the pathogenesis and validate experimental drugs. The gastric organoids interact between immune cells and epithelium to study the gastric pathophysiology of bacterial infection, gastric cancer, and gastric repair. Gene therapy in the field of gastroenterology and hepatology has served as a potential tool where several conditions lack effective therapy including non-resectable neoplasms of the liver, pancreas and gastrointestinal tract, chronic liver hepatitis unresponsive to interferon therapy, liver cirrhosis and inflammatory bowel disease [30].

Intestinal organoids provide an ability to mimic the intestinal immune system as the intestinal lumen is continuously in contact with foreign materials and microbes. Intestinal organoids incorporate microbiota and viruses that defends the growth of invading bacteria and breaks down food to assist nutrient absorption by intestinal epithelial cells. On addition to commensal bacteria, pathogenic bacteria for example Escherichia coli, Salmonella typhi, Cryptosporidium, are also incorporated into intestinal organoid systems to study and understand the effects of such pathogens on the intestinal epithelium [31].

Limitations on elucidating the surrounding cells of GI pathophysiology are found in organoids. Methods of culturing organoids are also suggested to improve as it failed to recapitulate the arranged structures observed in vivo. In recent researches, it was found that brain organoids can further be used to co-culture GI organoids to study the mechanisms underpinning digestive disorders [32].

4.1.2. Cystic fibrosis

Cystic fibrosis (CF) is an inherited life-threatening disorder. It causes severe damage to lungs, digestive system, and other organs in the body. It affects the cells that produce mucus, sweat and digestive juices, by making them thick and sticky. They then plug up tubes, duct, and passageways. CF is the most common monogenetic recessive disease and is caused by mutations in the Cystic Fibrosis transmembrane Conductance Regulator (CFTR) gene. More than 2000 alterations have been identified. F508del is cystic fibrosis' most common causative mutation, with 90% patients carrying at least one CFTR copy with this alteration. Every infected patient has two copies of defected gene, inherited from each parent [33].

Treatment for CF only cover a limited set of mutations. Therapies now available are prescribed to patients with the F508del mutation in both copies of CFTR gene. Though, this leaves 10% of CF cases, those carrying other mutations in CFTR gene, named ultra-rare mutation, without any approved medical treatment [34]. A therapy based on the use of organoids from CF patients with rare mutation is on track. As organoids are functional expression for individual genomes therefore, these cultures are incredibly useful for how genetic factors play their part in a particular disease [35].

ASC-based organoids have induced a great impact in the field of CF research. ASCs are progenitor cells found in epithelial tissues. It is an alternative for iPSCs for generating organoids. Because of the numerous characteristics they have high regenerative capacity. Their nature and function depend on position inside the body, to which they remain intact in-vitro. The main function of ASC is to maintain tissue homeostasis by retaining normal cell turn-over or repairing tissue after damage [36].

Largely, ASC-derived organoids can be generated within days or weeks and can be maintained for over a year. Also, they can be stored in biobanks for future reference. Patient-derived intestinal organoids recapture patient-specific characteristics, like it captures patient variation within identical mutation group or rare-mutation group by recapitulating genetic expression contributing to disease severity [36]. Out of many developed patient-derived organoid models, intestinal organoids, and Forskolin-induced swelling (FIS) assays offer numerous advantages, such as manipulation is not required for CFTR testing. As organoids do not require pre-incubation, swelling is completely dependent on CFTR gene. Also, vast manifestation can be predicted since assay can be performed in 96- and 384-format [37]. Studies have shown that intestinal organoids are powerful pre-clinical models as they harbor all kinds of CFTR mutation in infant-derived organoids. They are highly helpful to study individual relations between CFTR genotype, expression, and function in-vitro. They also exhibit outstanding accuracy for separating drug responders from non-responders [38].

Also, CRISPR-based editing of mammalian genomes was applied to intestinal organoids for mutation modification and correction. Healthy intestinal organoids respond by immediate swelling due to fluid secretion by the forskolin-activated CFTR channels. On the contrary, organoids derived from CF patients with the mutation do not expand their surface area. So, the researchers used CRISPR to improve and correct the CFTR mutation by co-transfection of a repair template. Thus, the utilization of CRISPR/Cas9 genome editing toolkit to amend the CFTR locus by homologous recombination in organoid culture system of CF patients leads to betterment of drug screening and development of a precise medication [39].

However, there are limitations such as ASC-derived organoids are relatively small and limited by the diffusion distance of oxygen and nutrients. Also, the maturation into functional in-vivo like tissue is a major challenge. These drawbacks need to be eliminated to take full advantage of organoids. At present, scientists' focus is on ASC-derived airway organoids as pulmonary failure is the main cause of death in CF patients. Main objective is to develop affordable drug screening techniques and personalized medicines that enable CFTR restoration, accessible for patients with any mutation at any geographical location and with no ethical barriers [36].

4.1.3. Hepatitis

Hepatitis is an inflammatory disease of the liver. It is commonly caused by a viral infection whereas it also includes autoimmune hepatitis or hepatitis that occurs as a secondary result of medications, drugs, toxins, and alcohol. Autoimmune hepatitis refers to the disease which occurs when our body makes antibodies against our liver tissue [40]. Organoids are 3D cell culture systems with the objective to decipher diverse research questions related to hepatic development and detoxification, regeneration and studies related to metabolism of liver disease modeling. The particular organoid model of hepatic progenitors was derived by stepwise differentiation of cells from iPSCs and co-cultured with MSCs and HUVECs [40].

The organoids explore the viral infection and the pathophysiology of the disease. Tissue biopsies from patients are directly used for production of disease-specific organoids. Therefore, disease-specific mutations

into liver organoids derived from healthy donors have become rapidly enabling to researchers and thus, are helping them to readily investigate mutation-related mechanisms and clinical phenotypes [41]. Gene therapy strategies developed for hepatitis include gene silencing by harnessing RNA interference, transcriptional inhibition through epigenetic modification of target DNA, genome editing by designer nucleases and immune modulation with cytokines [40].

However, organoid cultures fail to recapitulate the complex network between different body systems. No such studies have been reported explaining about the mechanism and process of studying hepatitis virus – infected hepatocytes by using liver organoids [42]. Liver organoids have proved the most powerful culture system in modeling liver diseases and are becoming an increasingly viable option for patient-specific therapeutic strategies in personalized liver medicine.

4.1.4. Alzheimer's disease

It is a progressive neurodegenerative disease that destroys memory and other important mental functions. Brain cells and their connections degenerate and die. Alzheimer is the most common cause of dementia worldwide which accounts for up to 80% of dementia cases. It mainly has two pathologies: β-amyloid plaque deposition and neurofibrillary tangles of hyperphosphorylated tau. Diagnosis is based upon clinical presentation. Doctors conduct tests to assess memory impairment. Brain scans are conducted and tests for other mental functions are also performed, like cerebrospinal fluid and positron emission tomography combined with several biomarkers. Treatments are based upon symptomatic therapies. There is no cure for Alzheimer's disease (AD) so far. Clinical trials are in progress to reduce production of pathological agents within the brain [43]. Due to lack of advanced experimental in-vitro models that truly recapitulate intricacy of human brain, research on human brain growth and neurological disease is limited. But brain organoids have a great potential for future discoveries [44].

Generation of brain organoids is carried out in numerous ways. Guided brain organoids can be generated from hPSCs through embryoid body formation in a culture media, along with extrinsic factors such as ECM and exogenous differentiation signals. hPSCs exhibit endless self-renewal capability and they can also differentiate towards mesoderm, endoderm, or ectoderm. Unguided brain organoids generated from hPSCs self-organize and assemble in the absence of extrinsic factors. Studies have illustrated that 3D brain organoids systems have a great potential to demonstrate and recapitulate key features of AD pathophysiology [45].

AD brain organoids are being used as a platform to evaluate the potency of pharmacological agents in advancement of disease research [45]. As hundreds of organoids can be generated simultaneously, the possibility of developing drug screening increases [44]. Because of organoid systems, a lot of growth has been made in modeling the disease and in evaluating potency of several drugs, like γ-secretase inhibitors to reverse AD-related phenotypes. Unguided brain organoids due to their differentiation pattern have shown capability in modeling cell-lineage variety in entire brain development. Whereas guided brain organoids are used to seize, and research processes associated with particular brain regions, involving the hippocampal loss in AD [45].

Since several genetic variants related to heightened risk for disease is related to non-coding regions of genome, a better model is required to study AD. Integrated with a broadening genome editing toolkit such as CRISPR/Cas9, developments in genome engineering methods have advanced our knowledge of central

nervous system. Thus, organoids with modified genome are possible hope for significant development of disease modeling in neurological diseases such as AD [46-48].

Along with all the achievements there are some limitations that needed to be eliminated, such as lack of vascularization in the brain organoids prevent further development of the neuronal cells which prohibits the survivability of organoids [44]. Other limitation is related to aging as it is one of the main causes of AD. In hPSC derived organoids, aging is achieved by numerous genetic alterations, but it changes overall cellular transcriptional profile [45]. With all the developments and achievements in the field of organoids in near future we can expect cure for such neurodegenerative diseases like AD.

4.1.5. Zika virus

This deadly disorder is primarily known to infect the placenta, amniotic fluid, blood, and brains of fetuses [14]. A dorsal-forebrain specific organoid generated from hPSCs using SpinΩ, is exposed to two different strains of the virus. This demonstrated fatal disorders such as microcephaly and infection in neural progenitor cells (NPCs) [10]. The infected neural progenitors released infectious Zika virus (ZIKV) particles [14]. ZIKV was hence known to be prone to NPCs, like radial glia cell (RGCs) and ortho RGCs (oRGCs), on exposure of the forebrain organoid [49]. According to Xu et al. [50], it was hypothesized that the inhibition of AXL receptor tyrosine kinase protein (highly expressed in RGCs and oRGCs), could confine the transmission of this virus. This was confirmed by eliminating AXL using genome editing in hPSCs. The cerebral organoids formed exhibited no difference in the ZIKV infection. It was concluded that AXL inhibition has no effect on ZIKV infection [49].

The ZIKV infection was also seen to influence apoptosis in progenitor cells, diminished their proliferation and increased the lumen size in ventricular structure, finally causing decrease in neuronal cell-layer volume. This study was proved to be consistent with clinical cases of the infected patients [14]. From the analysis done in several papers, it has been evident that the regulation of the response of an innate immune receptor, Toll-like-Receptor 3 (TLR3), is responsible for the infection. The regulation of genes, such as Netrin 1 (NTN1) and Ephrin type-B receptor 2 (EPHB2), is also responsible for causing ZIKV infection. However, further studies are necessary for the clarification on TLR3-ZIKV relationship [14]. According to a latest study, DNA methylation is also described as a consequence of the disease. This phenomenon of disease modeling is also beneficial for drug testing. For instance, drugs like hippeastrine hydrobromide and amodiaquine dihydrochloride dehydrate have already been in use as experimental means [51].

4.2. Study on Coronavirus disease of 2019 (COVID-19)

The end of 2019 saw the outbreak of a pandemic, which began from Wuhan, China and continued to spread throughout the world. The COVID-19 or coronavirus disease is a disease which is caused by the novel coronavirus or the SARS-CoV-2, which seems to directly infect the respiratory system, gastrointestinal tract as well as the cardiovascular system. The disease has a potential for widespread infection, which can also have other symptoms like dry cough, fever, weakness, muscle pain or in more severe conditions, acute respiratory distress syndrome (ARDS) or septic shocks [52, 53].

As described above, coronavirus can cause gastrointestinal infections. Researchers have been using intestinal organoids to study the molecular mechanism of SARS-CoV-2 in the intestine. These organoids summarize the in-vitro cellular and molecular factors of the intestinal epithelium. The intestinal organoids are typically polarized, so that their apical surface is placed on the luminar surface of the organoid, as they could enable an easy access to both the surfaces when grown as a 2D organoid derived monolayer [54].

A team of Japanese researchers in Tokyo have been successful in creating miniature bronchi which would conduct air into the lungs. The miniature organoid cultured from undifferentiated cells in the human body, naturally known as stem cells, can be used to study the novel coronavirus and further may help in developing drugs for COVID-19. The research is taking place at Kyoto University's Centre for iPS Cell Research and Application or CiRA. They have successfully created bronchial organoids with a diameter of 0.0002 meter from commercially available cryopreserved human epithelial cells. It was noted that it takes roughly 10 days to cultivate. It was then infected with the pneumonia-causing virus, and tested upon by camostat, which is a drug often used for treating pancreatitis. Such an experiment was found effective in reducing the viral load in organoids.

The team has provided knowledge that the miniature bronchi contains four types of cells as well as a receptor for COVID-19. The organoid is now testing the efficacy of other medicines, including anti-flu drug called Avigan also known as favipiravir. It is also expected that the miniature organ will fulfill as a better model for analyzing anti-viral drugs potency. A member of the team also included that since, developing a drug for COVID-19 is an urgent task now, so they chose a method which is simple and does not take time [52].

In another paper from Life Science Institute at the University of British Columbia in Canada, it was seen that the Angiotensin converting enzyme 2 (ACE2) receptor acts as an entry gate for SARS-CoV-2 (coronavirus). The organoids are created to study the COVID19 symptoms more elaborately. It was then found that due to the infection in the outer layer of the intestine by the virus, the gut related COVID-19 symptoms like diarrhea are shown. Organoid studies can further help in studying how the virus lives inside cells and therefore, how it can be blocked from entering. Thus, preventing the virus from finding the ACE2 entry gate is probably the most rationale therapy possible for COVID-19 [55].

In another study, it was seen that few patients exhibited neurological symptoms, which indicates that SARS-CoV-2 is also neurotropic. It also indicates that the virus can directly interact with neurons with the help of 3D cerebral organoids. It was reported that the virus infects cortical neurons and not neural stem cells, in organoids. Though, there is a low ACE-2 expression in the brain organoids, SARS-CoV-2 infects and causes neuronal cell death. Therefore, cerebral organoids can contribute as a suitable model system to study SARS-COV-2-CNS interactions as well as to study the neurotropic phase of SARS-CoV-2 [56].

Novoheart, an international stem cell biotechnology company, has constructed what is referred to as "heart-in-a-jar". This system carries stem-cell engineered human ventricular cardiac organoids placed in a hollow pump, which is an appropriate caricature of the human heart. These organoids have been utilized by certain companies to screen the potency of drugs, like hydroxychloroquine and azithromycin, on the human heart. For instance, the working method of these drugs and how they can give rise to arrhythmias has been revealed. Apart from drug targeting, these miniature organs aid in the study of the direct effects of coronavirus on the heart. It has been discovered that the virus can induce fatal disorders like myocarditis (myocardial inflammation) in the heart [57].

Hence, organoids act as a model system to procure all the information for researching the pathogenesis of SARS-CoV-2, both in a fundamental research context and during drug development for COVID-19.

4.3. Drug screening

One of the primary applications of these 3D miniature organs is to predict the effect of several drugs, chemicals. and biological agents on humans. Animal testing and cell screening are considered to be less

accurate for testing drug toxicity [58]. It had also been observed that 2D cultures were not efficient for drug diffusion kinetics.

Anthony Atala and his team at The Wake Forest Institute of Regenerative Medicine had previously studied and researched on the concept of "organ-on-a-chip" (OoC) platforms. Following this, similar models for tissue-on-a-chip and on-chip disease models using the principles of microfluidics and microengineering had been put forth [59].

Anthony Atala, the director of the Wake Forest Institute of Regenerative Medicine had recently collaborated with Army Edgewood Chemical Biological Centre (ECBC), on the Ex-vivo Console of Human Organoids (ECHO) project. They have provided a study regarding the concept of "body-on-a-chip" (BoC), wherein the system comprised of bioprinted organoids for testing toxic effects of drugs [60]. In addition, the sustenance and proper functioning of the system was ensured by the circulation of a nutrient-filled liquid, which also served as a medium for drug injection. Fluidic device technologies were utilized to incorporate the tissues of liver and heart organoids accurately into infusible devices. The liver and the heart were associated with the lung at ALI. The major challenges were the maintenance of the integration of the system and the inter-tissue interactions. The real-time data observation was constantly monitored by biosensors [59].

Another study performed at Cincinnati Children's Hospital, also demonstrated a similar system which was based on the idea of a "living gut", which consisted of liver, pancreas, and biliary ducts as miniature organs. It was considered to be an effective tool to study the effects of gene variations and various other factors on human development and on drug targeting. Presently, the gut organoid formed were found to lack HES1 gene. The experiment began by testing basic medications on the system [61].

Another positive aspect of BoC systems is that indirect or side-effects of a drug on other organs can be estimated. For instance, when a cancer drug had been tried on a multi-tissue organ-on-a-chip system, it portrayed a side effect on the heart. This was surprising, as these observations deviated from the expected results, which posed a negative effect on the lungs. Another fine example is a diabetes drug, Rezulin (Troglitazone), which was approved by the Food and Drug Administration (FDA), USA, in 1997 ignoring the danger it posed to the liver. 63 diabetic patients had suffered fatalities due to liver failure; voluntary report-based statistic which reflects 1-10% of actual fatalities (Los Angeles Times); on consumption of the drug and its withdrawal was then announced in 2000. Some news articles even highlighted on the cardiac risks that the drug posed. On conducting animal trials, it was seen that they overweight, discolored hearts, which suggested cardiac toxicity. But animal testing was not expected to predict the exact effects of the drug on human body. Hence, the results were ruled out even though diabetics had a higher risk of developing congestive heart failure [62]. Very recently, Atala and his team had conducted trials of Rezulin drug on their miniature organ system embedded on a chip, and the drug portrayed liver toxicity within 2 weeks [57].

Apart from this, scientists at the Hubrecht Institute have been successful in creating reptilian organoids, derived from snakes. The preliminary attempt of generating the reptilian (venom) organoid was using the eggs of Cape Coral snake. The organoids were able to secrete active toxins (from vesicles in venom glands) that have been identified in snake venom. The toxins secreted are known to serve as an important source for preparation of new medicines and therapeutics, including anti-venom. A plus point noted during the culture was the indefinite growth shown by them. The study also showed evidences regarding the neurotoxins that are produced, which caused blockage in nerve firing. Single cell RNA sequencing showed four venom-expressing cell types [63]. However, there are currently very few annotated genomes as compared to snake organoid genomes. The snake venom organoids contained proliferating progenitors and cell heterogeneity is

also maintained in the venom composition [64]. Their intention is to develop organoids from 50 other reptilian vertebrates and snake species [65].

Gene manipulation also plays a major role in boosting large scale generation of drug precursors [66]. The methodology has proved to be useful in drug assistance for high throughput screening (HTS), i.e., for detecting response of bulk cell lines or tumors [67]. Besides, even human gene therapy is refined by genome editing, especially for polygenic disorders [66]. CRISPR technology has lately been used to determine if the difference in sensitivity of RAS oncogene in colorectal organoids, was due to the Epidermal growth factor receptor (EGFR) and Mitogen-activated protein kinase (MEK) inhibitor drugs. CRISPR is utilized to show the affirmation of the relationship between gene alterations and subsequent drug sensitivity [67].

Drug screening for cancer in patient-derived cells holds assurance for drug discovery and personalized oncology as well.

4.5. Personalized oncological medication

Personalized medication is a beginning to overcome the limitations of the traditional medicine system. The drugs and treatments developed are tested and verified on large populations and are prescribed using statistical averages. Modifying such medications to each person's unique genetic makeup is the promising idea behind personalized medicine. Organoid culture system has already been utilized in the medical science for drug screening, now it represents a turning point in the successful discovery for personalized medicine.

4.5.1. Prostate cancer

Prostate cancer is a very common cancer occurring in men. It usually refers to prostate adenocarcinoma, where adeno means gland and carcinoma refers to uncontrolled growth of cells. Therefore, prostate cancer is a tumor or growth that originates in the prostate gland.

Organoid culture of lymph node carcinoma of the prostrate (LNCaP) and C4-2B cells in Matrigel was performed and culture media was allowed to stand for 14 days. It was found that both LNCaP and C4-2B cell lines formed glandular structures presenting organoids. Thus, it was found that LNCaP and C4-2B cells can form cancer organoids under the defined organoid culture conditions [68].

For organoid development, fresh tumor tissue with metastatic prostate cancer from 25 patients was derived. From these an overall success rate of 16% (4/25) was obtained. For further planned studies on tumor microenvironment, a cytology smear was performed to confirm the presence of tumor cells in the culture and thus, cancer associated fibroblasts were isolated and propagated separately.

The organoids were engrafted as patient derived organoid xenografts (PDOXs) using NOD/SCID gamma mice and re-passaged in-vitro as organoids from PDOXs. The pathology of the patient's metastatic tumor and their organoids and PDOXs was classified as neuroendocrine prostate cancer based on morphology of the tumor. Patients with de novo small cell neuroendocrine prostate cancer or castration-resistant neuroendocrine prostate cancer (CRPCNE), were previously treated with platinum-based chemotherapy whose prognosis was poor and gave no known effective therapies. However, the recent therapeutic advances of PDOXs gives us more effective results and opens the gateway towards advanced high-throughput drug screening [69].

The histone EZH2 is an epigenetic modifier overexpressed in prostate cancer. Therefore, patients with CRPCNE were treated with EZH2 inhibitor to explore the activity of drugs. Successful results have come out when CRPCNE organoids were treated with EZH2 inhibitors. Reduction and a preferential decrease in the viability of CRPC-NE organoids took place [70].

4.5.2. Ovarian cancer

Ovarian cancer (OC) is lethal gynecologic cancer and one of the leading causes of female cancer death. OC refers to any cancerous growth that begins in the ovary. Mostly OCs start in the epithelium, or outer lining of the ovary.

It is mostly diagnosed in later stages, common symptoms are abdominal pain, loss of appetite, weight loss, etc. It remains undetected until it has spread within the pelvis and stomach, i.e. advanced stages, and metastasis [71]. Most OC cases show epithelial phenotype. About 90% cases are OC. This cancer has many histological subtypes. Recently it is subdivided into 2 types, TYPE 1 consists of low-grade serous carcinoma, mucinous carcinoma, endometrioid carcinoma, etc. and TYPE 2 consists of high-grade serous carcinoma, undifferentiated carcinoma and carcinosarcoma [72]. Since pathobiology is poorly known for OC due to lack of appropriate study models, proper treatment and diagnosis techniques are undeveloped even if detected in early stages with surgery and chemotherapy prognosis. Survival period is maximum 5 years and still survival rate is as low as 47%. But early detection is difficult due to non-specific symptoms. Since treatment options are still limited, scientists are working on new therapeutic options. One such option is organoids; it is an emerging field in oncology organoid cultures are applied to various patient-derived samples for disease screening and personalized medicines [72].

In order to generate patient-specific organoids, patients' tumor biopsies are dissociated into fragments and cells, then implanted in a 3D ECM platform and cultured with supplementary growth and signaling factors, optimized according to the particular cancer type [73]. So far, nine OC-derived organoid lines are developed from high-grade serous carcinoma, mucinous, endometroid carcinoma, and even from early-stage or borderline tumors [72]. Patient Epithelial OC-derived organoids generate the disease's cellular and molecular phenotypes, also captures the mutational profile of the initial tissue. They have high potential to recapitulate the genomic constitution of the primary tumor. Epithelial markers such as cytokeratin 8 were also expressed in the organoids as they were positive in primary tumor. Epithelial OC-derived organoids also demonstrate tumor-specific sensitivity to clinically applied chemotherapy [73].

Also, CRISPR/CAS9 techniques of genome engineering are also being employed in organoid culture systems. By doing so gene expression, RNA behavior, epigenetic changes or mutations can be modified easily. Genome engineering establishment in organoids makes it relatively easier to introduce a double strand break at any location in the genome, insert or delete nucleotides or entire gene sequence. Thus, makes modeling of tumorigenesis and modifying cancer driver genes in cancer organoids less hectic [74].

Nevertheless, with all the developments there are certain shortcomings in organoid culture techniques which needs to be addressed in future. For instance, tumor-derived organoids lack stroma, blood vessels, etc., and organoid culture system is costly [72]. Despite all the limitations, organoid culture platform might have promising potential in drug discovery and personalized medication.

4.5.3. Lung cancer

The need for genetic and architecture-specific response of every individual had driven the formation of Patient-derived xenografts (PDXs). These were in use initially due to their appropriacy for all cancer models and for exhibiting a positive response among lung cancer patients through high-throughput studies. They were also utilized for generation of xenograft models composed of subcutaneous (s.c) implant models and implantation under the renal capsule. However, they were considered as an overpriced choice for large drug screening, were less successful and resource intensive and hence not adopted for clinical use [75, 76]. Cancer

cell lines were not approved for use because they lacked maintenance in heterogeneity and 3D organ structure [77]. In contrast, PDXs maintained genetic character of the cancer for up to 14 passages. In place of PDXs, orthoxenografts, involving orthotopic tumor transplantation, were also introduced.

Orthoxenografts were also used for development of tumor models in mice, which utilized the growth of human lung cancer cell lines in bronchioalveolar region of the right lung. The implantation was done through i.b (intrabronchiolar) injection. Fresh tumor suspensions were also implanted intrabronchially. The i.b tumors grew more than the ones that had previously been inoculated s.c. Nonetheless, the i.b tumors remained confined to the right lung. A second model was created where the injection was given by i.t (intrathecal) route, into the pleural space but it was all the same as the i.b model [75, 76].

The differences in genotype and phenotype of each patient, and failure of PDXs and orthoxenografts led to the concept of personalized medicines for lung cancer. It involves the generation of lung cancer organoids, formed from cancer cells, which have a tissue architecture similar to primary lung tumors and maintain genomic alterations of the original tumors during in-vitro expansion. These genomic alterations are essential for selecting and developing cancer drugs and therapeutics as well as for generation of biobanking for individual patients. For instance, response of Breast cancer (BRCA-2)-mutant organoid to Olaparib, EGFR-mutant organoid to erlotinib, EGFR-mutant/MET-amplified to crizotinib. Previously used therapies, including EGFR mutations Anaplastic lymphoma kinase fusions (molecular-targeted) were generated using cancer cell lines. Others like Programmed death-ligand 1 (PD-L1) were derived by the expression of biomarkers [77].

Recently, lung cancer organoids have been generated using airway organoid culture, but it was shown to have some limitations. Furthermore, it is evident from a new study that iPSCs obtained from NSCLC cell lines maintain the NSCLC-associated methylation and transcriptional pattern of the oncogenes and tumor suppressing agents [78].

4.6. In-vivo transplantation and regenerative medication

Organoids have a bright future with all the advancements in stem cell technology. Maybe it will be an alternative for organ transplantation. According to a stat, 2 lac patients die of liver failure or liver cancer annually in India, about 10 - 15% of which can be saved with a timely liver transplant, but due to lack of organ donor they die. Also, in the United States 1.12 + lakh men and women register as organ donors annually still 20 patients die each day waiting for an organ transplant. This increasing shortage of human organ donors has driven research scientists to examine other options such as xenotransplantation. But there are many scientific and ethical issues emerging from xenotransplantation technologies [79].

So, the use of stem cell technology to develop human organoids is a less ethically fraught alternative. In addition to this, existing in vitro tests and animal models do not sufficiently reflect the complexity and specificity of the human immune system. Even novel humanized animal models have limitations in their systemic reactions [80].

Scientists are using 3D technologies to develop mini organs such as the liver, placenta, stomach, lymph nodes, small intestine, kidney, salivary gland, prostrate, eyes, heart, inner ear and even brain. Also use of genome engineering for more precise use of modified genome in organoid system is enabling generation of human disease models and highly efficient regenerative medicine [81, 82].

Organoids are a promising apparatus to replicate key functional and structural characteristics of human organs. Recent modifications in organoids to model the liver, biliary tract, and pancreas have the potential to

advance regenerative medicine. Being able to engineer transplantable tissues in a dish would fundamentally change the way biomedical research and clinical practice works. Regenerative medicines are already being utilized for several diseases such as Inflammatory bowel disease (IBD), Junctional Epidermolysis Bullosa, etc. [83, 84]. IBD consists of two major GI diseases: ulcerative colitis and Crohn's disease. Although a considerable advance has been achieved in the treatment of IBD, there is a specific population of patients that are refractory to the established treatments, including the biological agents. Studies have suggested mucosal healing for improving the prognosis of difficult-to-treat patients, which represents the proper and complete regeneration of the damaged intestinal tissue and is considered really very important. In this concern, organoid-based regenerative medicine may have the power to improve the success and potential of mucosal healing in refractory IBD patients, and also improve their long-term prognosis. So far, researches have shown that hematopoietic stem cells and MSCs may be beneficial for IBD patients through their transplantation or transfusion. Modern stem cell biology has added intestinal stem cells (ISCs) as a new emerging variety of organoids. It has been shown that ISCs can be grown in vitro as organoids and that those in-vitro cultured organoids can be employed as donor cells for transplantation researches. Further studies using mice colitis models have shown that ex-vivo cultured organoids can engraft onto the colitic ulcers and reconstruct the crypt-villus structures. Such transplantation of organoids may not only stimulate the regeneration of the difficult-to-treat ulcers that may last in IBD patients, but may also reduce the risk of developing colitis-associated cancers. Transplantation of organoids may become one of the alternative therapies for refractory IBD patients. Many stem cell graftings are being ascertained for other diseases as well [85, 86].

Chronic kidney disease (CKD) has risen as a worldwide healthcare crisis. CKD regularly prompts end-stage renal infection, for which patients require either hemodialysis or kidney transplantation for survival. In any case, both renal substitution treatments are restricted. The mortality rates in patients on dialysis stay a lot higher than those in general, while transplantation is constrained by the deficiency of organ donors and there is a requirement for deep-rooted immunosuppressive treatment. Thinking about the expanding commonness and the yearly rate of Chronic kidney disease, researchers are working on new therapeutic options [87] .

For CKD, regeneration of lost nephrons with human kidney organoids derived from iPSCs is recommended to be a desirable potential therapeutic possibility. For this purified kidney organoids are transplanted beneath the kidney capsules of immunodeficient mice to test their safety and maturity. Kidney organoid grafts sustained and survived for months after transplantation and also became vascularized from host mouse endothelial cells. Nephron-like structures in grafts appeared more mature than kidney organoids in vitro but remained immature compared to the neighboring mouse kidney tissue, also not as organized as adult mammalian kidneys. Stromal expansion was observed in the stroma of transplanted kidney organoid grafts in the mice which was filled with vimentin positive mesenchymal cells, along with stromal expansion chondrogenesis, cystogenesis was also observed in long term. Transcriptomic reprogramming is induced in extended-term maintenance of culture after kidney organoid transplantation. This research implies that kidney organoids derived from iPSCs may be transplantable but techniques to upgrade nephron differentiation and purity. So, that they can be applied in humans [87].

The use of organoid platforms has led to developments in in-vitro organogenesis and disease modeling, and regenerative medicine. It has created possibilities for the development of innovative new medications. With the currently available vast techniques of bioengineering methods, it is possible to broaden the utility of organoids with improved control over external suggestions and with a remarkable opportunity to control and manipulate cellular behavior [79].

4.7. Developmental biology

The generation of organoids applies principles of developmental biology, such as, directed differentiation, morphogenetic processes, self-assembly of cells and recapitulation of organogenesis. As described above, organoids can be derived from all three germ layers. It is the pluripotent stem cells (iPSC and ESC), which drive differentiation into the 3D organoids. The specific combination and particular concentration of patterning signals and growth factors are responsible for activation of cell differentiation. The main signaling pathways involved in the development are Wnt, Retinoic acid (RA), FGF and transforming growth factor (TGF-β)/ bone morphogenetic protein (BMP) pathways [88]. Besides, two different approaches having to do with differentiation and organization of cells in culture. The first approach indicates towards organ-specific progenitors derived from iPSCs by exposure to various factors. After culturing, the cells self-organize into specific organ components. The second approach suggests the use of iPSCs that are strained to form cellular aggregates mimicking early preimplantation of embryo [89].

After organoid culture, the differentiation into mesoendodermal cells occurs when PSCs are exposed to activin A and for endodermal cells occurs due to high levels of activin A. The combined Nodal and Wnt activation leads to occurrence of gastrulation and the inhibition causes neuroectoderm formation. The neuroectoderm differentiation either generates differentiated neurons (cerebral region), in presence of RA or optic cup formation (retinal epithelium), in presence of fetal bovine serum, Shh and Wnt. After the formation of the three germ layers, viz. endoderm, mesoderm and ectoderm, the patterning occurs along the anterior-posterior embryonic axis. This is controlled by spatio-temporal levels of Wnt, FGF, RA and TGF-β/BMP [88]. Transcription factor caudal-type homeobox2 (Cdx2) is responsible for promotion of the posterior endodermal patterning, while the anterior endoderm patterning is dependent on the inhibition of BMP signaling. The mid and hindgut generation is promoted by Cdx2 expression which is caused via activation of Wnt and FGF signaling. The foregut patterning relies on combined action of inhibition of BMP signaling as well as activation of FGF and Wnt, causing suppression of Cdx2 and expression of [sex determining region Y]-box2 (Sox2). Foregut on exposure to RA forms gastric spheroids which further form antral organoids. On exposure to activated Wnt signaling, formation of gastric spheroids which results in formation of corpus organoids. TGF-β/BMP inhibition acts on foregut to form anterior foregut, finally forming respiratory lineages [88].

The consecutive activation of Wnt followed by FGF signaling causes the patterning of anterior and posterior mesoderm, further promoting formation of ureteric and metanephric mesenchyme respectively. Ureteric patterning is promoted by activation of RA, whereas metanephric patterning is promoted by inhibition of RA. The development of 2D endodermal culture into 3D spheroids occurs by exposure to Wnt and FGF. Few such examples are: FGF is considered essential for nephrogenesis in kidney organoids, EGF is required for maintenance of gastric and intestinal organoids, whereas FGF and Shh are used in combination for in vitro lung epithelial development. Endoderm-mesoderm interactions are important for early patterning and epithelial-mesenchymal interactions are essential for later patterning of the organoids in vitro. Mesenchyme is crucial for tissue morphogenesis plus the mesodermal cells are necessary for organ bud-like structures for organs like pancreas and liver [88].

Organoids may be considered as substitutes for cell types like blood vessels and neurons. Moreover, the idea of varied combination of the components of the endoderm, mesoderm, and ectoderm during organogenesis, in addition to the self-organization ability of cells, is utilized for organoids under culture [88]. Organoids are also valuable for the study of genetic and epigenetic defects in developing fetuses and correcting them [66]. CRISPR/Cas9 systems are well-known to correct heritable mutations, including

rectification of Myosin Binding Protein C3 (MYBPC3) mutation or even β-thalassemia mutation [90]. Till date, there have been studies that have suggested the rectification of gene mutations using tripronuclear (3PN) and two-pronuclear (2PN) zygotes, by bringing CRISPR/Cas9 into play. The first such implemented study was the correction of β-thalassemia mutation [91], followed by HIV-resistance mutation [92], hypertrophic cardiomyopathy [93], to name a few. This technique, though helpful in the study of developmental processes, may lead to rise in many ethical complications [61], and even chances of immune rejection [67].

The above stated concepts can be applicable for modeling diseases developed in fetuses during pregnancy. Ultimately, organoid studies may provide as a blueprint for developmental biology along with evolutionary studies [94].

5. PROSPECTS AND SUBSEQUENT LIMITATIONS

To study disease, human development and drug therapies, the use of 2D cell cultures has been around for a long time. Research has recently been abandoning them in favor of, more lifelike 3D constructions like organoids, capable of self-organization and self-renewal [95]. In vitro 2D cultures have been limited by flat, plastic and physiologically aberrant environments. In comparison, the 3D culture of organoids can more closely mimic natural, physiologic processes including stem cell differentiation, cellular movement, and cell-cell interaction. Furthermore, these miniature organs have also filled in for animal models. Organoids reduce experimental complexity, are amenable to live imaging techniques and can provide for more accurate and relevant models. In addition to delivering an actual response as organs in-vivo, they also minimize the ethical constraints posed by the animal models. Despite this, animal testing may dispense the researchers with intact models consisting of entire organ systems. However, organoids combine with other technologies such as testing drug toxicity through OoC system or testing gene and cell therapies by transplanting organoids, which are two further applications in development. Interconnected organoid systems, like OoC, are proving to be a reality in recent times. Although, a true BoC does not exist, researchers have succeeded in creating proximate systems [95]. Organoids are also considered to provide an insight in the study of the development of the human embryo, where they are believed to be faithful in recapitulating various tissue types and their functions. Recent studies have also substantiated the practicality of future therapies like organ donation techniques [94].

Many complications lie in this sphere of stem cell research in defiance of its propitious future. In the first place, organoids only contain epithelial layer without tissue microenvironment, such as immune system and nervous system. Secondly, complete maturation to adult organs or tissues is a bottle neck required to be addressed. The efficacy of organoids in cell replacement therapies is being placed under doubt due to lack in their robustness, reproducibility, and scalability. Challenges to the use of organoids also include lack of vascular and neural inputs, limited standardization of growth method, limitations in the physiological accuracy of the tissue architecture and absence of increased interstitial pressure [4]. Another drawback involves bridging the gap between the demand and supply for organ transplantation [6]. Researchers are seeking to overcome such challenges and with time standardized protocols should be feasible.

Most organoids are known to be capable of undergoing extensive expansion in culture while maintaining their genomic stability making long time storage (biobanking) and high-throughput screening possible [4]. Moreover, the utilization of genome editing in the process of 3D bioprinting of organoids has modified the approach towards modelling diseases and developing personalized medicines. Genome engineering has also been applied to organoid systems for inducing or correcting mutations in order to

elucidate pathological conditions. Both CRISPR and organoid technology have shown sudden recent development. Hence, their combination provides a great scope of study [62]. In fact, lately a study on generation of four-dimensional (4D) bioprinted structured has been put forth, by GE Healthcare [6]. 4D bioprinting generally refers to 3D bioprinting which involves printing of environmentally responsive structures consisting of tissues and organs. It can be categorized into mainly 3 types, viz., shape change, size change and pattern change, which can assess the effects of variation in physical forces and cell shapes [96].

The ongoing pandemic of the novel coronavirus is an eminent evidence of the efficiency of organoid technology. Numerous laboratories are making use of organoid studies to determine the virus-host interactions and the viral pathogenesis. According to an article by Nature, several researchers have revealed the direct effect of this virus on blood vessels, kidneys, liver, intestines using the corresponding organoids [97]. With effective endurance in overcoming the challenges and through analyses to comprehend the results, it is expected that organoid culture will become a vital tool for both basic and applied research.

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