International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Organoids: A New Approach for Clinical Trial in a Dish

Lakshmi Sravani Kolnada[1], P. Veerashbabu[2]

[1]Gokaraju Rangaraju College of Pharmacy, Bachupally, Kukatpally, Hyderabad-500090
Email: *lakshmisravanikolnada[at]gmail.com*

[2]Gokaraju Rangaraju College of Pharmacy, Bachupally, Kukatpally, Hyderabad- 500090
Email: *pratap.veeresh[at]gmail.com*

Abstract: ***Organiods, sometimes referred to as organs in a dish, have transformed the disciplines of biological and medical research, and the technology has proven to be a great animal model substitute in preclinical investigations, immunotherapy, and stem cell biology. Organoids are three-dimensional (3D) cell culture systems that are sometimes referred to as "mini-organs" because they closely resemble certain important multicellular, anatomical, and even functional traits of actual organs.Organoids are effective tools for studying individual illnesses and developing specialized treatments. This cutting-edge technology has increased the likelihood that medications will be translatable for use in preclinical therapies and mimicked the complexity of organs, proposing a variety of methods for tissue engineering, drug development, diagnosis and regenerative medicine. There are numerous technologies that are connected to organoids, which may lead to wonders in the future.***

Keywords: organoids, mini-organs, cutting-edge technology, tissue engineering, drug development

1. Introduction

Organoids are three-dimensional (3D) cell culture systems that are sometimes referred to as "mini-organs" because they closely resemble certain important multicellular, anatomical, and even functional traits of actual organs. A 3D culture of various cell types, derived from tissue explants, tumors, stem cells, or other progenitor cells is referred to as an organoid. Under controlled conditions, these cell types self-organize and differentiate into functional cell types to take on the complexity, anatomy, and physiology of an organ or other body structure. A tumoroid is a term used to describe an organoid that resembles a primary tumor. The study of organoids has transformed the realms of biology and medicine, and the technology has proven to be a highly effective replacement for animal models in preclinical research. Here, we explain what organoids are and how they have evolved into common research tools over time. Various organoids that resemble tumors and bodily parts, including the brain, lungs, intestines, liver, kidneys, and retina, are described in depth, along with their properties and uses [1].

The search criteria used to write this review article was history of organoids, how are organoids made?, characteristics of organoids, advantages of organoids, disadvantages of organoids, organoids intelligence, genetics of organoids, organoids and nanotechnology and application of organoids.Specific criteria used for searching review article was how is computers linked with organoids, involvement of CRISPR tool in organoids and how is nanotechnology linked with Organoids, most of the information was taken from frontiers, nature journal, technology networks and national library of medicine.

2. History

The first attempt at in vitro organism regeneration was published in 1907 by Henry Van Peters Wilson, who demonstrated that separated sponge cells may self-organize to rebuild an entire species. [3]

Since then, several research teams have tried to scale that mountain, starting with the dissociation and reassembly of chick embryos and frog pronephros to better comprehend the mechanisms of dissociation and differentiation. [2]

In 1981, the initial isolation of pluripotent stem cells (PSCs) from mouse embryos [4] marked a significant turning point in stem cell research. However, human blastocyst-derived embryonic stem cells weren't initially identified and grown until 1998. Then, using reprogrammed mouse and human fibroblasts, iPSCs were created, and cell culture technology has since continued to advance by imitating the microenvironment found *in vivo*. [2]

The finding that adult intestinal stem cells can generate 3D intestinal organoids in Matrigel that self-organize and develop into crypt-villus structures while expressing the G protein-coupled receptor 5 (Lgr5) was the key to the breakthrough. The first report on creating a 3D organoid culture from a single ASC was this one, and it paved the way for numerous subsequent organoid studies in other systems, such as mesendoderm [RM1] [BO2] (e.g., stomach, liver, pancreas, lung, and kidney) and neuroectoderm (brain and retina) using either ASCs or PSCs (Figure 1). [2]

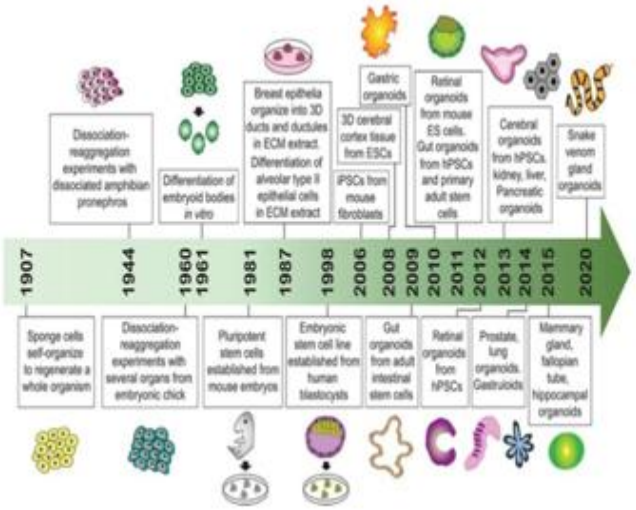

Figure 1: Timeline of how organoid cultures have evolveda list of significant research achievements and studies that helped build various organoid technologies.[Credits:MedChemExpress]

2.1 How are Organoids Made?

Organ-restricted adult stem cells (AdSCs) and pluripotent stem cells (PSCs), which include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are two different types of stem cells that can be utilized to make organoids. From tissues, organs, or cryopreserved organoids, suspensions of cells or their fragments can be made by mechanical or enzymatic dissociations. These cells or tissue fragments are initially seeded in differentiation fluid on low-attachment plates or culture containers for 5-7 days to produce 3D cellular aggregates known as spheroids. Spheroid structures can be produced in roller bottles, tiny bioreactors and conventional suspension culture. These spheroids are then put into liquid ECM, matrigels or agarose-based gels. Depending on the progenitor cells being used and the organ or tissue shape that needs to be rebuilt, protocols are altered. In other cases, the differentiation process is avoided by suspending and planting the cells obtained from organoids or tissue fragments directly into liquid ECM. The gels are polymerized at 37 °C to produce a 3D culture. The necessary cell types are then encouraged to develop in this culture using organoid-specific expansion and maturation medium, resulting in organ-like structures. For instance, the addition of the growth factor EGF stimulates the growth of epithelial and tumor organoids, but the addition of fibroblast growth factor 10 is necessary for the growth of stomach, breast and liver cancer tumoroids. [5-6]

Typically, fully-grown, functional organoids are created in the maturation medium, which is renewed twice a week, after 30 to 60 days (perhaps even longer). It is possible to keep this mature organoid for up to a year, or about 100 days, by passing the organoids and switching the maturation medium once or twice every 10 days. Organoids are gently broken into tiny pieces for 15 to 20 minutes with moderate cell dissociation agents, followed by vigorous pipetting throughout the passaging procedure. After the maturation media has been removed, this is done. After a succession of centrifugation and washing steps, the final organoid pellets are obtained, and the organoid production process is then restarted with them by resuspending them in liquid ECM.

Organoids can be frozen, and from these frozen organoids, totally detached organoids can be produced[5-6]. A wide range of specialized media and media supplements, as well as other materials like transwells, well-plates, or matrices, are commercially available to support the various systems of organoid technology. The protocol can be easily adjusted to the target organ or body part that needs to be replicated.

The three components that make up organoids are:
The physical characteristics of the culture environment, the activation or inhibition of key signaling pathways, the starting cell type and state.

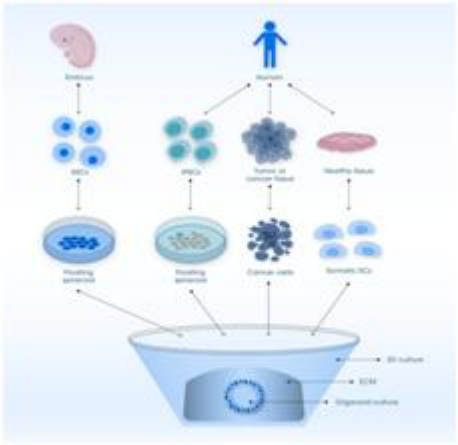

Figure 2: shows a schematic representing how organoids are produced from human embryos, organs, or tumours. Abbreviations: induced pluripotent stem cells(iPSCs); extracellular matrix(ECM); embryonic stem cell(ESC); stem cell(SC); three-dimensional(3D).
[Credit: Technology Networks.]

Cultural Approach of Organoids

Submerged culture
The most popular method for cultivating organoids is submerged culture, which was created by the Clevers.[7]In this process, cells or cell clusters are embedded in extracellular matrix (ECM) gels, which are then combined with the cells to create a dome in a culture plate. Medium is then added to completely enclose the dome. Basement membrane extract, Matrigel, and Geltrex are examples of popular ECM gels that provide structural support and ECM signaling. Epidermal growth factor (EGF), FGF2, FGF10, Wnt3A, Noggin, R-spodin-1, prostaglandin E2, N-Acetylcysteine, Nicotinamide, Y27632, A-8301, and SB202190 are the primary ingredients of the medium for the submerged culture.[8] When producing organoids from various tissues, the medium composition varies, and which particular elements should be added or deleted typically depends on the needs of the tissue from which they originate for the relevant signal or hormone.[9]

Ali culture
Utilizing ALI technology, which Ootani*et al*., initially suggested for the production of pancreatic and gastrointestinal tract organoids, it is possible to create the 3D

culture conditions for organoids.[10] The mechanically separated tissue pieces were homogeneously imbedded in collagen gel; the combination was set flat in an inner culture dish with a porous membrane beneath; and the top of the mixture was exposed to air. An outer culture dish was filled with the media. To address the needs of the organoids in this system,nutrients and growth agents are diffused from the medium in the outside dish into the inner dish. [11] Since ALI cultures are directly exposed to oxygen, they receive more oxygen than submerged culture methods. Later, they developed more patient-derived tumoroids utilizing the ALI technique, including some uncommon tumour forms as bile duct ampullary adenocarcinoma.[12]Organoids and stromal cells can both grow the same culture plate as part of the ALI culture system. It's important to note that organoids created using this method can retain functioning tumor-infiltrating lymphocytes (TILs), which can mimic the intricate tumour immunological milieu.[13]

Organoids on chip

A microfluidic cell culture device called a "organ-on-a-chip" enables precise control of the biophysical and biochemical parameters for cell development and replicates inter-tissue and multiorgan interactions as well as cellular and microenvironmental settings.[14]Organ-on-a-chips, which are utilised for disease modelling and to research the function of related organs, come in a range of designs to replicate analogous organs *in vitro*.[15]

Although organ-on-a-chip and organoids differ fundamentally, organoids-on-a-chip produced by combining organoid and organ-on-a-chip technologies can make up for these differences and serve as more useful preclinical models for simulating key characteristics of target organ tissues. Unlike the meticulously constructed organ-on-a-chip, cells in organoids-on-a-chip randomly and spontaneously self-organize into 3D structures. [16] Organoids of the liver, pancreas, gastrointestinal system, brain, and heart have been created in a number of recent research. Based on PDMS chips, Cho *et al.*, [17]created a brain organoids-on-a-chip. This system might boost oxygen delivery and encourage nutrient/waste exchange to lower organoid cell death. Notably, this culture technique was capable of producing fully developed brain organoids and could be used to track the growth of the entire human brain. According to a recent study, the organoid-on-a-chip technology can maintain the intestinal microbial barrier and produce a hypoxic gradient in the lumen of tiny intestinal organoids.[18]

In addition to the aforementioned culture techniques, hanging drop and rotating culture techniques may be used to create 3D systems for organoids. The mixture of cells and droplets of particular media were suspended from a plate using the hanging-drop method, which combined surface tension and gravity.[19]By continuously rotating or moving the cells, the rotational culture method—used to create retinal and brain organoids—can prevent cells from settling and increase the intake of nutrients and oxygen.Patient-derived glioblastoma organoids were created by Jacob *et al.*,[20] preserving the histological, genetic, and immunological characteristics as well as a portion of the microvasculature.Organoids are comparable to their parental cells in that they multiply and simulate their unique biological characteristics. Organoids can also self-renew and self organized include different cell types, execute specialized functions, and develop spatial structures akin to in vivo organs. As a result, organoids are useful models for investigating illness onset, development, and progression.

2.2 Characteristics of Organoids

Organoids can be characterized using a number of widely used techniques. These methods include high-resolution microscopy, histopathology, immunofluorescence, and bulk gene expression testing.[21]

For initial characterization, a light microscope might be utilized. A crucial stage of in vitro cell culture activity is cell counting. The accuracy and reproducibility of following experiments are ensured by using the right organoid concentration. Organoid counts are helpful for monitoring proliferation rates, experiment seeding, and assay preparation.[22] Organoids can be counted using a coverslip, a glass slide, or a hemocytometer.[23] Organoids can be counted as drops on a glass slide if they are too large for the hemocytometer.[24]

The number and shape of organoids can also be determined using high-resolution microscopy. Organoid counts can be recognized visually before studies are conducted and for experiments that need to be normalized to controls.[25] Analyzing 8-bit binary images of full organoid drops allows one to calculate the number and size of organoids.[26]Alternately, whole-well z-stack images can be used to record all organoids in a well using a transmitted light inverted microscope equipped with a motorized X/Y scanning stage. The morphology of organoid cultivation can then be investigated by taking measurements to put a number on elements like form and area. [27]

Particularly when it comes to maximizing growth factor concentrations for the creation of organoids, morphological analysis is a useful technique.[28] For evaluating morphology and differentiation, a number of techniques are available, such as whole-mount staining, FFPE, and brightfield image analysis. [27]

Organoids' cell vitality can be assessed using luminescent cell viability tests. To study samples' interiors and make sure that necrosis is not present in an organoid's core, histologists can embed organoids.[29] Tissue-specific cell markers found in the sample as well as immunofluorescent imaging can be used to determine the organoid's stage of differentiation.[29] The cell population of an organoid can also be characterized using flow cytometry and bulk gene expression tests. The quantitative polymerase chain reaction (qPCR), transcriptome analysis, and, more recently, single-cell RNA sequencing (scRNA-seq) are examples of these gene expression assessments.[30]

The organoids are available for their downstream applications once they have been characterized.

Examples of Organoids

A decade of groundbreaking research has produced human-derived organoids that imitate tumours, embryos, and a variety of tissues and organs, some of which are mentioned here.

Brain organoids

Organoids created from human PSCs may replicate cellular and regional interactions as well as the development of blood vessels in the brain. These organoids are made up of many cell lineages. To investigate the development, function, and pathology of the human brain, brain organoids that resemble certain parts of the brain or combinations of many brain regions and/or cell lineages are used as representative models. The pathophysiology of Parkinson's disease, the mechanisms of Zika virus infections in the brain, aberrant inhibitory neurons in autism spectrum disorder and epilepsy, and gene alterations in microcephaly have all been studied using human PSC-derived brain organoid models.[31]

Lung organoids

The infectious mechanisms of the respiratory syncytial virus and influenza virus have been effectively studied using human airway organoids. The SARS-CoV-2 infection and subsequent medication screening were also studied using them as a typical experimental model. To understand SARS-CoV-2 pathophysiology and aid the creation of novel treatments, a number of additional alveolar organoids originating from various cell types have been created. These organoids imitate certain locations or a mixture of many sites in the lungs. Prior to being given to people, more than 1,000 medications, including 25-hydroxycholesterol, remdesivir, and camostat, underwent testing in lung organoid models to see how well they inhibited SARS-CoV-2.[32]

Intestinal organoids

Single intestinal stem cells are used to form organoids that mimic the intricate, asymmetrical architecture of the gut, including the crypts and villi. Intestine function and homeostasis are significantly influenced by the highly specialised epithelial barrier. Organoids have made it possible to successfully identify biological targets that can be drugged as well as small molecule medications that act as regulators in the structure and function of the barrier epithelia. For research into the infectiousness of the Omicron version of SARS-CoV-2, the intestinal epithelia for the small intestine and colon that were reconstructed using organoids provide hope.[33]

Liver organoids

Models of liver fibrosis, lipid metabolism, and genetic stability in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis have been successfully created using multicellular liver organoids from human primary hepatic stellate cells and iPSCs. The differentiation of progenitor cells, or PSCs, into hepatocytes and liver buds has been done using liver organoids. To create mature hepatocytes, they could be implanted into mice with a high engraftment efficiency, or they could even be vascularized into living, breathing livers. These results suggest that organoids may play a part in liver transplants in situations when an organ has been damaged or failed.[34]

Kidney organoids

In recent years, advanced bioengineering methods have produced very complex kidney organoids, enabling the modelling of a number of illnesses, including polycystic kidney disease, cystic fibrosis, renal cell cancer, and viral infections. 19% of medicines that fail in clinical trials do so due to nephrotoxicity. As a result, the preselection potential of numerous widely used drugs, including gentamicin, aspirin, cisplatin, and penicillin G, has recently been investigated in kidney organoids. In kidney organoids infected with SARS-CoV-2, the therapeutic benefits of human recombinant soluble angiotensin-converting enzyme 2 were evaluated. Additionally, congenital nephrotic syndrome organoids produced from patient-derived iPSCs are used to research gene mutations and individualised treatments.[35]

Tumour organoids

Utilising original cells or cancer cell lines for the lung, liver, kidney, and heart in culture, recent advances in organoid technology have recreated the tumour microenvironment. With their morphologies, histopathologies, epigenetic processes, and genetic profiles preserved, organoids from many different cancer types may be frozen or grown for a very long period, allowing researchers to investigate the development of cancer and the clinical characteristics of tumours. To support immunotherapies and precision medicine in light of the clinical heterogeneity in cancer patients, large-scale biobanking of patient-derived organoids (PDOs) has been carried out for cancers of the brain, breast, cervical, colorectal, gastric, head and neck, kidney, liver, ovarian, and pancreatic.[36]

Retinal organoids

Retinal organoids have been successfully created from retinal stem cells and separately from ESCs, and they are capable of closely resembling the visual cycle and reactions to light in healthy eyes. Retinal organoids have been successfully used to simulate gene alterations implicated in the aetiology of the retinal diseases retinitis pigmentosa, Leber congenital amaurosis, retinoblastoma, and glaucoma. Human retinas might perhaps benefit from the transplantation of pigmented photoreceptor cells made from hiPSCs to treat age-related macular degeneration.[2]

Advantages of Organoids

The upsides of using organoids over traditional cell culture techniques or spheroids are covered below.

Individual-level modelling:

Organoids are created from lone people or patients to offer cellular, morphological, and other scientific data. Even tumoroids maintain the histological and genetic characteristics of the original tumours or malignancies from specific people. An individual's illness aetiology or overall therapy responses can be examined using tumoroids, numerous organoids tailored to specific organs, or multiorgan-on-a-chip.[37-38][2]

Cellular matrix or intercellular interactions:
ECM provides the primary structural organisation and permits cellular activities and communications for the healthy operation of biological tissues and organs. Cell-matrix interactions also play a role in these processes.[37] Spheroids, monolayer cultures, even co-cultures of two or more cell types cannot produce organoids, which allow interactions between several cell types and resemble complex structures resembling organs. Organoids give researchers a chance to investigate cell-to-ECM and cell-to-cell interactions in three dimensions.[2]

Efficient and high-throughout preclinical model:
Organoids may be used effectively to simulate disease processes and assess the efficiency of drug candidates and other therapeutic treatments for disorders since they have a higher similarity to in vivo systems than spheroids or conventional cell cultures. A large number of organoids may be produced from a little amount of human tissue, and multiple tests can be run in parallel on these organoids. As a result, the time required for drug screening and testing is shortened, and the discrepancies between in vitro testing and real patient responses are decreased, which lessens the financial burden associated with manufacture, preclinical testing, and clinical trials prior to the release of the pharmaceuticals into the market.[38]

Preservation of organ-like features:
The primary benefit of organoids over conventional cell culture techniques is the 3D spatial organisation of the cultured cells, which closely resembles their actual morphologies. Organoid development uses principles that are comparable to how organs naturally form when embryos develop into newborns to help progenitor cells differentiating and assembling into organ-like structures. Organoids, as opposed to conventional cell cultures or even spheroids, maintain the cellular and molecular processes of the cells. Because of these characteristics, organoids are good models for researching how organs grow and function as a whole as well as in specialised areas.[2]

Replacing animal models with specific species representations:

Human organoids can be used instead of animal models to study human organs, eliminating species-specific processes in the process and giving researchers the chance to conduct mechanistic research using a "human model" system.[2]

2.3 Disadvantages of Organoids

The vascular system is absent
Waste, nutrients, and oxygen are continuously exchanged across the blood vessels as a result of the dynamic character of the human body, maintaining cell survival. Organoids, however, are grown using static methods without a circulatory system. As the organoids continue to grow, the center's cells are unable to receive enough nutrition, and the exchange of waste products becomes difficult. Other factors that affect the vascularization of organoids include cytokine concentration, the kind of organoid, and flow rate. There are currently two primary techniques used by scientists to create vascularized organoids: "in vivo vascularization" [39] and "in vitro vascularization" [40]. Organoids are inserted into the appropriate animal model in order to establish a blood-vascular connection between the host and the transplanted organoid model. By using genetic engineering or the implantation of vascular cells, I produced in vitro organoid vascularization.

A medium for organoid culture:
Because matrigel provides organoids with an extracellular matrix, which enables the study of cell-matrix interactions, it is widely used to generate organoids. Matrigel is a substance created by Engelbreth-Holm-Swarm murine sarcoma cells and is composed of laminin, collagen, heparansulfate proteoglycan, and entactin. Matrigel is highly complex and contains more than 180 distinct proteins, despite these advantages.

The inability to properly characterize its composition makes it difficult to comprehend how it affects the structure and composition of organoids. Matrigel might not have all the necessary components for organoids to grow as well as they should. Furthermore, because of its animal origin, Matrigel cannot be used in therapeutic settings for people. Matrigel-independent organoid culture methods must be developed in light of these limitations. [42-43]

Immune cells and organs:
The immune system is made up of regulatory proteins, immune cells, and immunological organs that work together to protect the body from outside invaders. Together, immune and epithelial cells maintain the physiological balance of people. To research illness models, treatment efficacy, and drug assessment, it is difficult to establish a model of organoids and immune cells that interact and integrate. Current culture methods cannot support the co-existence of immunological and tumor cells in vivo. Tumor organoids with a functioning immune system will generate data that is clinically relevant, shortening the time between the lab and the hospital. [41]

2.4 Organoids Intelligence

OI is a young, interdisciplinary area that uses brain-machine interface technologies and 3D cultures of human brain cells (brain organoids) to build biological computing. Current brain organoids will be scaled up into intricate, long-lasting 3D structures filled with cells and genes related to learning, and they will be coupled with next-generation input and output devices and AI/machine learning systems.[44]

The astounding accomplishments of artificial intelligence (AI) range from writing poetry to identifying medical ailments. In many aspects, the brain still performs better than technology. For instance, brains are often stronger at processing complex information like visuals and can accomplish many activities at once. Additionally, human brains are far more energy-efficient than artificial intelligence and machine learning algorithms. In reality, due of its unsustainable energy consumption, silicon-based computing is rapidly hitting its processing capability constraints.[44]

We are developing biocomputers based on brain organoids, a type of brain cell culture, to get around these restrictions. In our ideal world, this strategy will enable us to harness the incredible processing power of the brain to drive ground-breaking 'biological' computers.[44]

To fully realise the promise of this new science, which we refer to as organoid intelligence (OI), the following main areas of endeavour are defined:[44] transforming present brain organoids into larger, more resilient structures that are loaded with genes and cells related to learning.[44]

AI/machine learning systems and future input/output devices connected to brain organoids

Creating novel models, algorithms, and interface technologies to interact with brain organoids, comprehend how they process information and learn, and process and store the enormous volumes of data they will produce [44] using a "embedded ethics" strategy to make sure OI evolves in a morally sound and socially responsible way.[44]

All about organoids intelligence.

An innovative, multidisciplinary scientific subject called "organoid intelligence" seeks to create a new kind of organic computing system. These next-generation biocomputers will use lab-grown brain organoids as the "biological hardware" to tap into the processing capacity of the brain. The organoid's neurological activity will be recorded by special equipment, and it will receive educational data from these devices. The AI algorithms and new learning modelswill facilitate these interactions. With less energy usage, this technology promises to bring about unheard-of improvements in computer speed, processing power, data efficiency, and storage capacity.OI may also increase our knowledge of how the brain develops, learns, and remembers information, which may lead to novel neurological and neurodevelopmental problems therapies. [44]

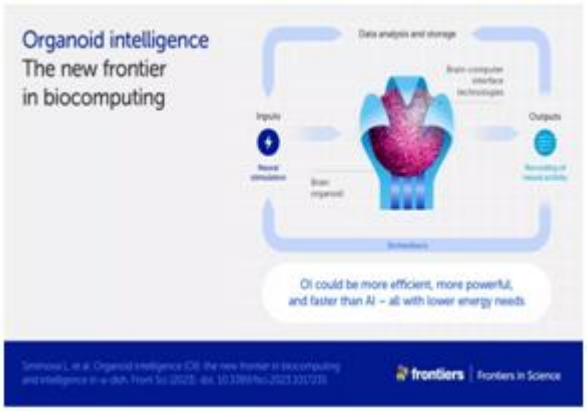

Figure 3: In this figure the data analysis and storage of organoids intelligence is shown.[credit: frontiers]

What are brain organoids and how they make good computer

Brain organoids are a type of cell culture created in a lab. Despite not being "mini brains," brain organoids share critical aspects of the structure and operation of the brain, such as the neurons and other types of brain cells that are essential for mental processes like memory and learning. Additionally, most cell cultures are flat, but organoids have a three-dimensional structure. This results in a 1,000-fold increase in cell density in the culture, enabling the development of many more connections between neurons.[44]

Hartung asserted that while brains are superior when it comes to learning, silicon-based computers are clearly better at managing statistics. For instance, AlphaGo [the AI that defeated the best Go player in the world in 2017] was trained using data from 160,000 games. It would take more than 175 years, or five hours each day, for one individual to play all of these games.

Brains consume less energy and are more effective at learning. For instance, the energy used to train AlphaGo is more than enough to sustain an active adult for ten years.The human brain can hold 2,500 terabytes (TB) of data, according to estimates. Silicon-based computers are getting close to their physical limitations since we can no longer pack more transistors onto a single tiny chip. Though very differently organized, the brain. Its approximately 100 billion neurons are connected by about 1015 connection sites. Over the technology we currently use, it has a significant power advantage. [44]

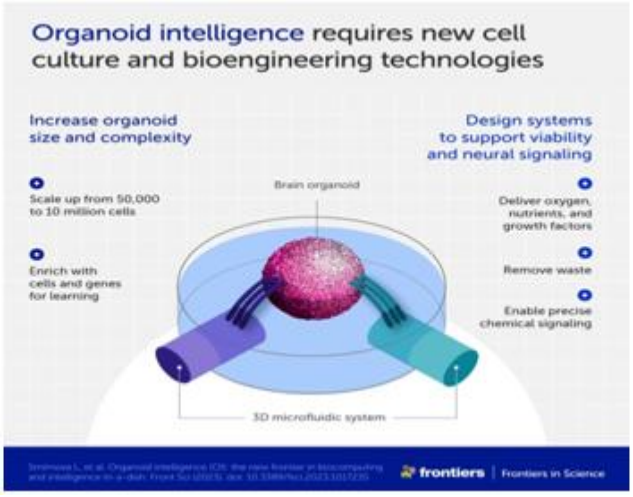

Figure 4: In this figure increase organoids size and complexity and design system to support viability and neural signaling is shown. [credit: frontiers]

Here is How we can treat neurological disorders with help of organoids intelligence.

Along with computers, OI has applications in the medical field. Thanks to a revolutionary technique developed by Noble Laureates John Gurdon and Shinya Yamanaka, adult tissues can now be used to make brain organoids. This suggests that scientists may develop personalized brain organoids from skin samples acquired from patients suffering from neurological diseases like Alzheimer's disease. Then, a variety of tests can be run to see how certain substances, medications, and genetic factors might affect these illnesses.[44]According to Hartung, we might use OI to investigate the cognitive impacts of neurological disorders. We might, for instance, contrast the development of memory in organoids derived fromAlzheimer's patients and healthy individuals in order to address relative

shortcomings. We may also use OI to check whether certain pollutants, like pesticides, harm memory or learning.

Figure 5: In the figure how organoids intelligence will advance medical reseach and innovations is shown.[credit:frontiers]

2.5 Organoids Genetics

Introduction:
Organoids have been genetically altered using a variety of techniques, launching a brand-new area of study called organoid genetics. With the use of these techniques, the genomic DNA sequence may be modified specifically. The target protein may alter specifically if the modifications are inserted into a coding sequence, which may shed light on the function of a particular residue or the protein as a whole in biological processes. In order to complete this procedure, it is important to take into account two key factors: the genetic instruments and the delivery strategy.

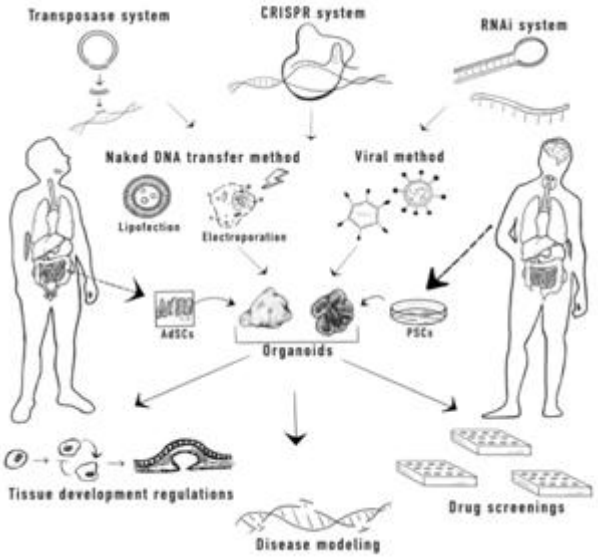

Figure 6: Adult stem cells (AdSCs) or pluripotent stem cells (PSCs) can both be used to produce organoids. AdSCs may be isolated from the tissue of origin and cultivated under the right circumstances to produce organoids that resemble the organ from where they were derived. Organoids made from PSCs are developed from cell lines of embryonic or induced pluripotent stem cells. The kinds of organoids made with AdSCs or PSCs, respectively, are shown on the left and right human figures. Different genetic engineering techniques, like as CRISPR/Cas, transposase, or RNAi, can be used to modify organoids. These tools might be distributed by a non-viral methods like electroporation or lipofection or a viral one like retrovirus, lentivirus, oradenovirus. The genetically edited organoids can be further utilized for various applications/fields of study including biological developmental models and translational/precision medicine, tissue development regulation, disease modelling, drug screenings. [credit:springer Link]

The tools which are used for genetic engineering of organoids
After settling on a mechanism of genetic delivery, the method of genetic editing must be considered. Because there are several publications comparing the various techniques for genetic engineering [45] we will only offer a brief overview of significant approaches used to genetically edit organoids: CRISPR/Cas9, retro/lentiviruses, transposons and RNA interference (RNAi).[46]

RNA interference(rnai) system
The RNAi system makes use of the cell's own mechanisms to block the production of particular genes. In this method, produced RNAs(siRNAs), hook up with the target gene's mRNAs to encourage translational silence or degradation, thereby suppressing the target mRNA's ability to express proteins. No prior genetic alteration is required for this technique to work in all mammalian somatic cells.[47]With a variety of vectors, including retro-/lentiviruses,adenoviruses, plasmids, and transposons, siRNAs can be introduced into cells.[46]

RNAi is only a knockdown technique, but it is less efficient and more prone to produce off-target effects.

Pros of RNA interference system include:
All mammalian somatic cells are affected, no prior genetic tampering is required; and multiplexing is conceivable.

Cons of RNA interference system includes:
Only works with knockdown, less effective, prone to off-target effects.

Transposing-based systems
The ability to transfer the target gene into the host genome for ongoing expression makes transposon-based systems like PiggyBac and Sleeping Beauty ideal choices for stable gene expression. PiggyBac and Sleeping Beauty both use the "cut-and-paste" strategy to "cut" the genetic sequence flanked by a certain terminal inverted repeat from one locus and "paste" it into another. [46] However, this random insertion does occasionally occur in an active gene, and this can have unexpected effects on the host cell.

Pros of systems based on transposons include:
Stable integration for long-term expression.

Cons of transposing-based systems include:
Difficult to implement on a wide scale, random insertion can disrupt transcriptionally active genes.

CRISPR-associated (Cas) systems
For sequence-specific editing in prokaryotic and eukaryotic cells *in vitro*, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems have been widely modified since 2012.[49] The

mechanism, which provides bacteria with adaptable resistance to bacteriophage infections, was first identified in bacteria.[50] The Cas9 endonuclease and single-guide RNA (sgRNA or gRNA), which uses a spacer sequence to connect to a complementary DNA sequence (protospacer sequence) and direct Cas9 to a particular target, were then added to the CRISPR/Cas9 system. For the Cas9; gRNA complex to introduce a double-strand break (DSB), a DNA target must have both the protospacer sequence and the protospacer adjacent motif (PAM). A wide range of applications are made possible by the fact that the PAM sequence varies for the many Cas9 and Cas12a (Cpf1) endonucleases produced from various bacterial species.[51]The DSB can be repaired by either homology-directed repair (HDR), which necessitates a template for accurate, high-fidelity repair, or by non-homologous end joining (NHEJ), in which the blunt ends are re-ligated together, following cleavage by the Cas9 nuclease.[52] Fixed by HDR Researchers can insert particular sequence alterations into target genes by following a provided template.[53] The cell must be in the S phase of the cell cycle for the repair to take place, making this method ineffective. The amount of work required increases because each gene's homology arms must be added to the template plasmid before it can be copied. Alternately, DNA repair can take place by NHEJ-induced nucleotide insertions or deletions, which cause frameshift mutations and inactivate the target gene.[54]

NHEJ is not employed for precision targeted mutation since it is thought to be error-prone. However, in recent work by Artegiani *et al*. [54] the NHEJ was modified for the creation of quick knock-ins in diverse human organoids.[54] As knock-in DNA is cloned into a self-cleaving plasmid containing a non-human sequence that is recognized by sgRNA, the effort needed for homology arm cloning is eliminated. Even with TP53 suppression, which has been proposed to increase the efficacy of HDR in human pluripotent stem cells, the scientists were still able to demonstrate more effective knock-in generation than HDR. [54]

Increasing the effectiveness of various Cas enzymes to detect a wider range of PAM sequencesor almost totally remove the PAM sequence constraintare further future CRISPR/Cas9 development goals.[55][56] Base editors are produced by altering the Cas9 endonuclease by fusing inactive Cas9 nickase to cytidine deaminase. New techniques for producing exact base alterations in organoids were made available as a result.[57]

Pros of CRISPR-associated (Cas) systems.
Introduce specific modification to target sequence, multiplexing possible, ease of scalability.

Cons of CRISPR-associated (Cas) systems.
Susceptible to immune reaction, possible off-target effects.

Organoid genetics and organoid-based disease modeling are given a new platform by combining organoid technology with diverse genetic editing methods.

2.6 Organoids Testing the Toxicity of Nanotherapeutics Usinga Novel Method

Numerous industries, including agriculture, the consumer market, medicine, and other sectors have been transformed by nanotechnology. Widespread use of products based on nanotechnology has increased the frequency of these innovative formulations in the environment, raising questions about their potentially harmful impacts. The lack of efficient *in vitro* methods that could precisely assess their *in vivo* harmful effects prevents the deployment of formulations based on nanotechnology in clinical settings. Numerous investigations in two-dimensional *in vitro* cell cultures and animal models have demonstrated the harmful effects of formulations based on nanoparticles.[58] When used to assess nano-toxicity, these have certain associated drawbacks. The gap between current two-dimensional cell line culture and *in vivo* models is filled by organoid technology.[59]Organoids stand out from other testing platforms for assessing the toxicity brought on by nano-drug formulation due to their co-culture of various cell types, dynamic flow that mimics the movement of nanoparticles in biological systems, extensive cell-cell and cell-matrix interactions, and tissue-like morphology.[60] As a result, it replicates the actual tissue microenvironment, which makes it possible to research drug metabolism and the toxico-dynamics of innovative formulations based on nanotechnology.[61]The assessment of the toxicity of nano-drugs using organoids is still in its infancy. Only a small number of research have been done so far, but those that have demonstrated strong predictive value and statistically significant data association with therapeutic trials.They are trying to introduce organoids of the liver, lungs, brain, kidney, and intestine, as well as potential uses to assess the toxicity brought on by nanoparticles.[62]

2.7 Applications of Organoids Technology

Organoids in the field of regenerative medicines
The field of regenerative medicine may use organoids. Organoid systems and biofabrication techniques can be used to simulate complicated tissue or organ functions. Human inflammatory bowel disease and short bowel syndrome patients may benefit from organoid-based therapeutic therapies thanks to recent advancements in the development of intestinal organoids.

To restore intestinal epithelial function, a systematic technique is being developed. By generating the organoid within a complex three-dimensional framework to deliver functional tissues, organoids may be used as a source of intestinal regenerative medicine. Additionally, intestinal organoids may be reintroduced into the body for functional development.

Human colonic stem cells have been successfully transplanted into mouse colons in studies, demonstrating the viability of using intestinal epithelial organoid cells in a transplant-based therapy.

Human colonic stem cells have been successfully transplanted into mouse colons in studies, demonstrating the

viability of using intestinal epithelial organoid cells in a transplant-based therapy.[63-66]

Organiods used a personalised medicine.

Patients will receive the best possible care that is unique to them thanks to personalized medicine. Because organoid systems are formed from a single patient biopsy and the culture will show genetic similarities, they are a crucial tool in the development of customized medicine.

The long trial and error testing of treatments prescribed to the patient can therefore be replaced by tests of drug efficacy in organoid systems.

The approach has already shown success in determining unique cystic fibrosis treatment outcomes. The production of the CFTR protein, which is responsible for cystic fibrosis, is impacted by the CFTR gene mutation.

CFTR modulators aid in correcting the damaged protein, but different patient reactions have been observed. The effectiveness of CFTR modulators has been effectively assessed using intestinal organoids.

Since the outcomes were found to mimic *in vivo* medication responses, organoid systems can offer a more accurate method of anticipating patient response to cystic fibrosis treatment.[63-66]

Organoids used for cancer research

Currently, cancer development and treatment are modelled using organoid systems. Because there aren't any *in vitro* models that can faithfully reproduce the physiology of the original tumor, cancer research has been constrained.

In 2017, primary liver cancer organoids that preserved the physiological structure and gene expression of the original tumor were produced.

The results of *in vivo* transplantation in mice showed that the cancer-based organoids' long-term *in vitro* expansion still preserved the parent tumor's histology, as shown by the secondary tissues derived from the grafted tumors' sharing the parental line's chromosome counts and morphology.

Additionally, the cancer-based organoids were successfully used in a variety of medication sensitivity tests. The findings suggest that in the future, organoid systems that preserve the histology and gene expression of the original tumor can be used to predict medication sensitivity and a patient's particular response to cancer treatments.[63-66]

Human organoids used for modelling genetic disease.

Modeling human genetic illnesses and the creation of novel therapeutic approaches are two of the main uses of human organoids. For the first time, Hans Clever*et al*. created intestinal crypt organoids using adult intestinal stem cells.[67]Then, to examine cystic fibrosis (CF), a hereditary condition brought on by mutations in the CF transmembrane conductance regulator (CFTR), gut organoids were utilized. Thus, a rapid and reliable test for the measurement of CFTR function was developed, which will hasten the diagnosis of CF, functional research, medication screening, and personalized medicine approaches.[68]Following the successful creation of gut organoids, an increasing variety of organoids of various types were created and utilized to represent genetic diseases.

The creation of kidney organoids from a patient with polycystic kidney disease by Menendez *et al*. laid the groundwork for research into additional hereditary kidney diseases.[69] Recently, Fabry disease causes and potential therapy options were investigated using kidney organoids produced from hiPSCs with a GLA mutation. Retinal organoids have also been used to model genetic retinal disorders. Similar to this, cardiac organoids with knockouts for NKX2-5 and HAND1 have been used to replicate hypoplastic left heart syndrome, the most severe congenital human abnormality. In particular, the study of numerous neurodevelopmental genetic diseases has made extensive use of brain organoids. For instance, patient-specific cerebral organoids produced from iPSCs have been used to imitate microcephaly, a condition connected to CDK5RAP2 mutations that is challenging to reproduce in mice. Various other neurological conditions, such as Down syndrome and Alzheimer's disease, and PD, have also been modeled with brain organoids.[70-71]

Organoids used in process of drug discovery and in development of personalised medicine

Personal differences, unpredictable outcomes, and time-consuming pharmaceutical testing are commonly barriers to present medical understanding of human disorders. A specific disease-based 3D organoid has shown a lot of promise for medication testing.[72] Organoids made from iPSCs of patients with primary malignancies, infectious diseases, or developmental diseases show clinical traits resembling those of primary cancers and could be used as test subjects for new medications.[73] In 2017, Broutier et al. identified an ERK inhibitor as a potential therapeutic agent using human primary cancer organoids.[74]

The adenomatous polyposis coli (APC) gene, a negative regulator of the WNT pathway, was mutated in patients with familial adenomatous polyposis coli (FAP), leading Crespo et al. to produce colonic organoids from these patients.[75] Additionally, two medications that might be harmful to wild-type organoids were evaluated; nonetheless, they were effective in preventing the overproliferation of patient organoids. Instead of using cancer organoids, drug development teams have started using brain organoids. The congenital Zika syndrome shares characteristics with ZIKV-infected cortical organoids, such as a thinner neuronal layer and ventricular lumen dilatation. In several studies, virally infected brain organoids have served as testing grounds for potential antiviral drug candidates such duramycin, ivermectin, and azithromycin. High-throughput drug testing on intestinal organoids is common. [76]

For instance, Kenji Kozuka et al. successfully screened about 2000 chemicals for prospective medicines by cultivating small colonic organoids in 96-well plates. Another example is a strategy that OnurCil's team put up in relation to the creation of antidiarrheal medications.[77]

Preclinical research and medicine toxicity assessment are still subject to some limitations, and many drugs don't become toxic until late in the development phase or during clinical trials.[78] Organoids can be used to study the effects of novel medications since they behave similarly to physiological tissues.[79]Therefore, the creation of an organoid-based long-term toxicity screening model with human physiological characteristics is exciting. Organoids can be used to assess drug toxicity, such as negative effects on the liver, heart, and kidneys.

Skardal et al.'s[80] creation of an organoid system with heart, lung, and liver organoids in a single recirculating perfusion system in a shared medium system allowed them to assess the pharmacological effects and toxic responses of various medications on whole organs.[81]

Organiods on gene repair and transplantation therapy

By changing the target genes within the body's cells, gene repair addresses a number of illnesses, including cancer, diabetes, heart disease, and acquired immunodeficiency syndrome (AIDS). Gene repair is looking more and more promising since genetically altered Lgr5+ stem cell-derived organoids have been produced and successfully implanted into injured tissues.[82] Because of its great gene editing effectiveness, CRISPR/Cas9 technologies have recently been used for gene repair.[83] Schwank *et al*. discovered that gene repair was functional after the human CFTR mutation F508del was fixed with a CRISPR/Cas9-mediated homology-dependent repair system, presenting a possible gene treatment for CF patients.[83] The first successful CRISPR/Cas9 gene-editing example in human-derived organoids has been accomplished thanks to this therapy.[84] A more sophisticated technique known as CRISPR-based adenine editing (ABE) has been created since this CRISPR/Cas9-mediated homology-dependent repair method may result in potentially damaging off-target double-strand breaks. With the help of this technique, the function damaged by the CFTR mutation can be repaired. On-target base editing is made possible, including precise enzymatic conversion of A-T base pairs into G-C base pairs.[85] With this advancement, it is clear that gene editing may correct genetic defects in PDOs, quickly advancing gene repair into the clinical stage.[86]

For the treatment of patients with organ failure, organ transplantation is a crucial resource. There are still a lot of obstacles to overcome, particularly acute graft rejection and a growing lack of organ donors. Offering a viable foundation for autologous transplantation therapy are endlessly accessible *in vitro*-cultured organoids that have developed into potential donors. Researchers have worked hard to confirm the viability of organoid transplantation therapy in order to make it a reality.[87] When Watson *et al*. transplanted an intestinal organoid made from hPSCs into a host mouse, they discovered that the organoid had significantly expanded and matured.[88]In host rats with retinal abnormalities, McLelland*et al*. showed that transplanted sheets of retinal organoids may develop broad projections and synaptic connections. According to Chen *et al*., lung bud organoids displayed branching morphology and proximodistal specification at 5 months following ectopic transplantation that were comparable to those seen *in vivo*.Wang*et al*. created cerebral organoids and inserted them into a rat model of stroke. Following transplantation, they noticed decreased brain infarct volumes and improved neurological motor function. Notably, our team has demonstrated that human cerebral organoids can form subcortical projections in host mice and functionally integrate into neuronal circuits. A significant theoretical foundation for the investigation of transplantation therapy has been established by numerous further investigations on organoid transplantation.[89-90]

The therapeutic use of organoids will still be a significant therapeutic option to organ transplantation even though transplanted organoids are comparatively young compared to the host's natural organs due to their insufficient functional maturation and potential heterotypic cell interactions.[91]

3. Current Limitation of Organoids

Although organoids offer many potential uses, the current model is still rather rudimentary, and this technology's challenges are still being worked through by researchers. Organoids are first and foremost flawed reproductions. Only the epithelial layer of "tissues in a dish" lacks the original microenvironment's surrounding mesenchyme, immune cells, neurological system, or muscular layer. The co-culture of organoids with additional cellular components, such as immune cells, stromal cells, or neural cells, as demonstrated by the co-culture of PDAC organoids with mouse pancreatic stellate cells that differentiated into cancer-related fibroblasts andiPSC-derived intestinal organoids with a functional nervous system, are two potential solutions to this limitation.[92]

Despite these promising results, it is challenging to model the immunological milieu that surrounds a tumor. An intricate network of immune cells, including cytotoxic lymphocytes, tumor-infiltrating dendritic cells, regulatory T cells, tumor-associated macrophages, and myeloid-derived suppressor cells, make up the immune niche of tumors. The tumor immune microenvironment is constantly changing, and there may be variations between various tumor types as well as between different patients. A second challenge that must be overcome to maximize therapeutic potential is complete maturity. Thirdly, some organoid lines still have limitations that may be resolved by altering the culture medium.[92]

Fourth, cancer organoids typically grow more slowly than equivalent organoids from normal epithelial cells, likely contaminating normal epithelial cells in the process and promoting the growth of tumor organoids. To reduce the contamination from normal cells, this issue might be solved by optimizing the tissue extraction procedure. Fifth, given recent developments in the production of organoids produced from primary glioblastoma as an example, cultures of non-epithelial organoids need to be further investigated. Current organoids are primarily formed from epithelium. Finally, the growth factors or small chemical inhibitors in the culture media may have a major impact on the signaling pathways and gene expression in organoids, as well as their

sensitivity to drugs. To solve this issue, further effort is required.[92]

4. Conclusion

Human organoids have a tremendous deal of potential in clinical translational research despite the remaining difficulties, thanks to the benefits mentioned above and to the quick, ongoing technological improvement. Organoid technology has developed to include genetic manipulation, various omics and drug-screening analyses, and a variety of co-culture systems with viruses, bacteria, and parasites. The original full "laboratory life cycle" began with the isolation of patient samples and progressed to the establishment of organoids and their cryopreservation. This will speed up our study of human biology and allow us to validate hypotheses and models created from studies in animal model systems. Technologies and experimental techniques that were developed in other model systems can now be used to human organoid systems. Given the rapid technical advances in the field, we believe that human organoid systems will provide unprecedented opportunities to improve human health.

References

[1] Lehmann r, lee CM, Shugart,EC, et al. Human organoids; A new dimension in cell biology. MolBiol, CELL. 2019; MAY 1;30(10):1129-1137.
[2] Corrò C, Novellasdemunt L and Li VSW. A brief history of organoids. Stem Cell and Cancer Biology Laboratory, The Francis Crick Institute, London United Kingdom; Am J Physiol Cell Physiol2020; 319: C151–C165.
[3] Wilson HV. A new method by which sponges may be artificially reared. Science 1907; 25: 912–915.
[4] Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl AcadSci USA 1981; 78: 7634–7638.
[5] Corro C, Novellasdemunt L, Li VSW. A brief history of organoids. Am J Physiol Cell Physiol. 2020; 319(1):C151-C165.
[6] Xu H, Lyu X, Yi M, Zhao W, Song Y, Wu K. Organoid technology and applications in cancer research. J HematolOncol. 2018; 11(1):116.
[7] Driehuis E, Kretzschmar K, Clevers H. Establishment of patientderived cancer organoids for drug screening applications. Nat Protoc. 2020;15(10):3380-3409.
[8] Meran L, Tullie L, Eaton S, De Coppi P, Li VSW. Bioengineering humanintestinal mucosal grafts using patientderived organoids, fibroblasts and scaffolds. Nat Protoc. 2023; 18(1): 108-135.
[9] Wang Q, Guo F, Jin Y, Ma Y. Applications of human organoids in the personalized treatment for digestive diseases. Signal Transduct Target Ther. 2022;7(1):336.
[10] Ootani A, Li X, Sangiorgi E, et al. Sustained in vitro intestinal epithelial culture within a Wntdependent stem cell niche. Nat Med. 2009;15(6):701-706.
[11] Lamers MM, van der Vaart J, Knoops K, et al. An organoid derived bronchioalveolar model for SARS-CoV-2 infection of human alveolar type IIlike cells. Embo J. 2021;40(5):e105912.
[12] Neal JT, Li X, Zhu J, et al. Organoid modeling of the tumor immune microenvironment. Cell. 2018;175(7):1972-1988.
[13] Hong HK, Yun NH, Jeong YL, et al. Establishment of patientderivedorganotypictumor spheroid models for tumor microenvironment modeling. Cancer Med. 2021;10(16):5589-5598.
[14] Xue Y, Seiler MJ, Tang WC, et al. Retinal organoids on a chip: a micro-millifluidic bioreactor for long term organoid maintenance. Lab Chip. 2021;21(17):3361-3377.
[15] Huang D, Liu T, Liao J, et al. Reversed engineered human alveolar lung-on-a-chip model. Proc Natl AcadSci USA. 2021;118(19):e2016146118.
[16] Saorin G, Caligiuri I, Rizzolio F. Microfluidic organoids on a chip: the future of human models. Semin Cell Dev Biol. 2022;144:41-54.
[17] Cho AN, Jin Y, An Y, et al. Microfluidic device with brain extracellular matrix promotes structural and functional maturation of human brain organoids. Nat Commun. 2021;12(1):4730.
[18] Shin W, Kim HJ. 3D in vitro morphogenesis of human intestinal epithelium in a gutonachip or a hybrid chip with a cell culture insert. Nat Protoc. 2022;17(3):910-939.
[19] Timmins NE, Nielsen LK. Generation of multicellular tumor spheroids by the hangingdrop method. Methods Mol Med. 2007;140:141-151.
[20] Jacob F, Salinas RD, Zhang DY, et al. A patientderived glioblastoma organoid model and biobank recapitulates inter and intra-tumoral heterogeneity. Cell. 2020;180(1):188-204.
[21] H.Wu and B. d. Humphreys, "single cell sequencing and kidney organoids generated from pluripotent stem cells,"clin, j. am. Soc. Nephrol., vol. 15, no.4, pp.550-556,2020
[22] T. Grabinger et al., Ex vivo culture of intestinal crypt organoids as a model system for assessing cell death induction in intestinal epithelial cells and enteropathy. Cell Death Dis.2014; 5(5): e1228--e1228.
[23] Rothschild DE, Srinivasan T, Aponte-SantiagoLA,ShenX, and AllenIC. The ex vivo culture and pattern recognition receptor stimulation of mouse intestinal organoids. JoVE (Journal Vis. Exp).2016; 111: e54033.
[24] Francies HE, BarthorpeA,McLaren-DouglasA, BarendtWJ, and GarnettMJ. Drug Sensitivity Assays of Human Cancer Organoid Cultures. in Organoids: Stem Cells, Structure, and Function, K. Turksen, Ed. New York, NY: Springer New York, 2019; pp. 339–351.
[25] FujimichiY,OtsukaK,TomitaM, andIwasakiT. An Efficient Intestinal Organoid System of Direct Sorting to Evaluate Stem Cell Competition in Vitro. Sci. Rep., 2019; 9: pp. 1–9.
[26] Boonekamp KE et al., Long-term expansion and differentiation of adult murine epidermal stem cells in 3D organoid cultures. Proc. Natl. Acad. Sci., 2019; 116(29): 14630–14638.
[27] McCrayT,RichardsZ,MarsiliJ, PrinsGS, and NonnL. Handling and assessment of human primary prostate organoid culture. JoVE (Journal Vis. Exp.)2019; 143: e59051.

[28] UrbischekM,RannikmaeH,FoetsT, RavnK, HyvönenM, and de la RocheM. Organoid culture media formulated with growth factors of defined cellular activity. Sci. Rep., 2019; 9(1): 1–11.
[29] Dossena M et al. Standardized GMP-compliant scalable production of human pancreas organoids. Stem Cell Res. Ther., 2020; 11(1): 1–12.
[30] Mullenders J et al. Mouse and human urothelial cancer organoids: A tool for bladder cancer research. Proc. Natl. Acad. Sci., 2019; 116 (10): 4567–4574.
[31] Koo B, Choi B, Park H, Yoon KJ. Past, present, and future of brain organoid technology. Mol Cells. 2019; 42(9):617-627.
[32] van der Vaart J, Lamers MM, Haagmans BL, Clevers H. Advancing lung organoids for COVID-19 research. Dis Model Mech. 2021; 14(6): dmm049060.
[33] Serra D, Mayr U, Boni A, et al. Self-organization and symmetry breaking in intestinal organoid development. Nature. 2019; 569(7754):66-72.
[34] Takebe T, Sekine K, Enomura M, et al. Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature. 2013; 499(7459):481-484.
[35] Khoshdel-Rad N, Ahmadi A, Moghadasali R. Kidney organoids: Current knowledge and future directions. Cell Tissue Res. 2022; 387(2):207-224.
[36] Gunti S, Hoke ATK, Vu KP, London NR, Jr. Organoid and spheroid tumor models: Techniques and applications. Cancers. 2021; 13(4):874-891.
[37] Lehmann R, Lee CM, Shugart EC, et al. Human organoids: A new dimension in cell biology. MolBiol Cell. 2019; 30(10):1129-1137.
[38] Rossi G, Manfrin A, Lutolf MP. Progress and potential in organoid research. Nat Rev Genet. 2018; 19(11):671-687.
[39] van den Berg CW, Ritsma L, Avramut MC, Wiersma LE, van den Berg BM, Leuning D G, et al. Renal Subcapsular Transplantation of PSC-Derived Kidney Organoids Induces Neo-Vasculogenesis and Significant Glomerular and Tubular Maturation In Vivo. Stem Cell Rep. 2018; 10(3): 751–765.
[40] Homan KA, Gupta N, Kroll KT, Kolesky DB, Skylar-Scott M, Miyoshi T, Mau D, Valerius MT, Ferrante T, Bonventre JV, Lewis JA, Morizane R. Flow-enhanced vascularization and maturation of kidney organoids in vitro. Nat Methods. 2019;16(3):255-262.
[41] Campinoti S, Gjinovci A, Ragazzini R, Zanieri L, Ariza-McNaughton L, Catucci M, Boeing S, Park JE, Hutchinson JC, Muñoz-Ruiz M, Manti PG, Vozza G, Villa CE, Phylactopoulos DE, Maurer C, Testa G, Stauss HJ, Teichmann SA, Sebire NJ, Hayday AC, Bonnet D, Bonfanti P. Reconstitution of a functional human thymus by postnatal stromal progenitor cells and natural whole-organ scaffolds. Nat Commun. 2020;11(1):6372.
[42] Andrews MG, Kriegstein AR. Challenges of Organoid Research. Annu Rev Neurosci. 2022;45:23-39.
[43] Kozlowski MT, Crook CJ, Ku HT. Towards organoid culture without Matrigel. Commun Biol. 2021;4(1):1387.
[44] Smirnova L, Caffo BS, Gracias DH, Huang Q, Morales Pantoja IE, Tang B, et al. Organoid intelligence (OI): the new frontier in biocomputing and intelligence in-a-dish. Front Sci 2023:1017235.
[45] Cornu TI, MussolinoC,Cathomen T. Refining strategies to translate genome editing to the clinic. Nature Medicine. 2017; 415–423.
[46] Yin H, Kauffman KJ, Anderson DG. Delivery technologies for genome editing. Nature Reviews Drug Discovery. 2017;16:387–399.
[47] Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature. 2001;411(6836):494–498.
[48] Hudecek M, Izsvák Z, Johnen S, Renner M, Thumann G, Ivics Z. Going non-viral: the Sleeping Beauty transposon system breaks on through to the clinical side. Crit Rev BiochemMol Biol. 2017;52(4):355–380.
[49] Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl AcadSci U S A. 2012;109(39):E2579–E2586.
[50] Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;315(5819):1709–1712.
[51] Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cassystems. Science. American Association for the Advancement of Science. 2013;339(6121):819–823.
[52] BollenY,et al.How to create state-of-the-art genetic model systems: strategies for optimal CRISPR-mediated genome editing. Nucleic Acids Research. Oxford University Press, 2018; 6435–6454.
[53] Hustedt N, Durocher D. The control of DNA repair by the cell cycle. Nature Cell Biology. 2017; 1–9.
[54] Artegiani B, Hendriks D, Beumer J, Kok R, Zheng X, Joore I, Chuva de Sousa Lopes S, van Zon J, Tans S, Clevers H. Fast and efficient generation of knock-in human organoids using homology-independent CRISPR–Cas9 precision genome editing. Nature Cell Biology. 2020;22(3):321–331.
[55] HuH,et al.Long-term expansion of functional mouse and human hepatocytes as 3D organoids. Cell. Cell Press 2018a; 175(6): 1591-1606.
[56] Hu JH, Miller SM, Geurts MH, Tang W, Chen L, Sun N, Zeina CM, Gao X, Rees HA, Lin Z, Liu DR. Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. Nature. 2018;556(7699):57–63.
[57] GeurtsMH,et al. CRISPR-based adenine editors correct nonsense mutations in a cystic fibrosis organoid biobank. Cell Stem Cell. 2020; 26(4): 503-510.
[58] Abdel Fattah AR, Ranga A. Nanoparticles as versatile tools for mechanotransduction in tissues and organoids. Frontiers in Bioengineering and Biotechnology.2020.
[59] Adine C, Ng KK, Rungarunlert S, Souza GR, Ferreira JN. Engineering innervated secretory epithelial organoids by magnetic three-dimensional bioprinting

for stimulating epithelial growth in salivary glands. Biomaterials2018; 180: 52-66.
[60] Ahamed M, Akhtar MJ, Alhadlaq HA. Preventive effect of TiO(2) nanoparticles on heavy metal Pb-induced toxicity in human lung epithelial (A549) cells. Toxicology in Vitro 2019; 57: 18-27.
[61] Alépée N, Bahinski A, Daneshian M, De B, Fritsche E, Goldberg A, Hansmann J, Hartung T, Haycock J, Hogberg H, Hoelting L, Kelm JM., Kadereit S, McVey E, Landsiedel R, Leist M, Lübberstedt M, Noor F, Pellevoisin C, Zurich MG.State-of-the-art of 3D cultures (organs-on-a-chip) in safety testing and pathophysiology. ALTEX 2014; 31: 441-477.
[62] Alexis F, Pridgen E, Molnar LK, &Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. Molecular Pharmaceutics2008; 5: 505-515.
[63] XinarisC.,etal.Organoid Models and Applications in Biomedical Research, Experimental Nephrology and Genetics: Review2015; 130: 191-199.
[64] BroutierL.,et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nature Medicine2017; 23: 1424-1435.
[65] NakamuraT,& Sato T. Advancing Intestinal Organoid Technology toward Regenerative Medicine, Cellular and Molecular Gastroenterology2018; 5: 51-60.
[66] NoordhoeckJ.,et al. Intestinal organoids and personalized medicine in cystic fibrosis: a successful patient-oriented research collaboration. Current Opinion in Pulmonary Medicine2016; 22: 610-616.
[67] SatoT.,et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009; 459: 262–265.
[68] LiuJ.,et al. Functional Cftr in crypt epithelium of organotypicenteroid cultures from murine small intestine. Am. J. Physiol. Cell Physiol. 2012; 302: C1492–C1503.
[69] HofbauerP.,et al. Cardioids reveal self-organizing principles of human cardiogenesis. Cell 2021; 184: 3299–3317.
[70] TangXY.,et al. DSCAM/PAK1 pathway suppression reverses neurogenesis deficits in iPSC-derived cerebral organoids from patients with Down syndrome. J. Clin. Investig. 2021; 131: e135763.
[71] Miura Y et al. Generation of human striatal organoids and cortico-striatal assembloids from human pluripotent stem cells. Nat. Biotechnol. 2020; 38: 1421–1430.
[72] Dedhia PH, Bertaux-Skeirik N, Zavros Y & Spence JR. Organoid models of human gastrointestinal development and disease. Gastroenterology 2016; 150: 1098–1112.
[73] Drost Jet al. Sequential cancer mutations in cultured human intestinal stem cells. Nature 2015; 521: 43–47.
[74] Broutier L et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. Nat. Med. 2017; 23: 1424–1435.
[75] Crespo M et al. Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing. Nat. Med. 2017; 23: 878–884.
[76] Kozuka K et al. Development and characterization of a human and mouse intestinal epithelial cell monolayer platform. Stem Cell Rep. 2017; 9: 1976–1990.
[77] Cil O et al. Benzopyrimido-pyrrolo-oxazine-dione CFTR inhibitor (R)-BPO-27 for antisecretory therapy of diarrheas caused by bacterial enterotoxins. FASEB J. 2017; 31: 751–760.
[78] Vlachogiannis G et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. Science 2018; 359: 920–926.
[79] Katsuda T et al. Conversion of terminally committed hepatocytes to culturablebipotent progenitor cells with regenerative capacity. Cell Stem Cell 2017; 20: 41–55.
[80] Skardal A et al. Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. Sci. Rep. 2017; 7: 8837.
[81] Takasato M et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. Nature 2015; 526: 564–568.
[82] Huch M, BojSF,&Clevers H. Lgr5(+) liver stem cells, hepatic organoids and regenerative medicine. Regen. Med. 2013; 8: 385–387.
[83] Schwank G et al. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 2013; 13:653–658.
[84] Roper J & Yilmaz ÖH. Breakthrough moments: genome editing and organoids. Cell Stem Cell 2019; 24: 841–842.
[85] Geurts MH et al. CRISPR-based adenine editors correct nonsense mutations in a cystic fibrosis organoid Biobank. Cell Stem Cell 2020; 26: 503–510.
[86] Nie J &Hashino E. Organoid technologies meet genome engineering. EMBO Rep. 2017; 18: 367-376.
[87] Watson CL et al. An in vivo model of human small intestine using pluripotent stem cells. Nat. Med. 2014; 20: 1310–1314.
[88] McLelland BT et al. Transplanted hESC-derived retina organoid sheets differentiate, integrate, and improve visual function in retinal degenerate rats. Investig. Ophthalmol. Vis. Sci. 2018; 59: 2586–2603.
[89] Nie YZ et al. Human liver organoids generated with single donor-derived multiple cells rescue mice from acute liver failure. Stem Cell Res. Ther. 2018; 9: 5.
[90] Weiner AI et al. Mesenchyme-free expansion and transplantation of adult alveolar progenitor cells: steps toward cell-based regenerative therapies. Npj. Regen. Med. 2019; 4: 17.
[91] Garreta E et al. Fine tuning the extracellular environment accelerates the derivation of kidney organoids from human pluripotent stem cells. Nat. Mater. 2019; 18: 397–405.
[92] de souza n., Editorial, “methods of the year 2017:organoids,” Nature methods 15,no.1 (2018): 23.
[93] PiyaliKhamkat, vivekBarik, Bhakti BhusanBarik, SnehamoyeeMohapatra, RajatKar. Personalized medicine: An innovation in health-care system. Asian journal of pharmaceutical and clinical reseach.2022;4-9.

Author Profile

Lakshmi Sravani Kolnada pursuing bachelor of pharmacy from gokarajuranagaraju college of pharmacy Hyderabad. She has won awards for best

oral presentations from two international conferences and one national conference.

P. Veeresh Babu working as Associate Professor at GokarajuRangaraju College of Pharmacy, Hyderabad. He has bestowed with Sri. J. J. Rao Gold Medal by Andhra University, Best Researcher Award by INSO and Research Excellence Award by InSc. He has 42 publications and 30 presentation to his credit. He has guided 8 Ph.D students.