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Future direction towards human in-vivo organoid transplantation

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Abstract

Stem-cell derived organoids possess the potential to offer an unlimited source of transplanted tissues which would aid in overcoming concerns related to shortage of viable organs. Furthermore, organoids have proven to be exceptional in ex-vivo models for toxicity studies, drug screening and disease modelling due to their ability to respond to stress by expressing injury biomarkers from specific cell populations. The advancement in prolonged culturing of the organoids has allowed comparatively enhanced complexity and maturity of brain organoids. Hence, brain organoids have become important tools in studying how neuropsychiatric and neurodevelopmental diseases such as schizophrenia or autism spectrum disorder affect the mechanism of brain cell interactions. Moreover, organoids are utilized to test the hypothesis whether most lung diseases are due to stem cell dysfunction and if in the occasion of stem cell defect, new cell types could be the target for drugs hence helping in finding treatments to diseases such as cystic fibrosis. Ongoing research is trying to come up with methods of transforming and transplanting cells or tissue that could serve as a cure for certain diseases leading to the development of novel therapeutic tools. Although organoids have several benefits, most organoids that have been yielded exhibit partial components of corresponding tissue. Furthermore, regulating cell type, spatial organization and cell-cell, cell-matrix interaction have collectively become a challenge. For further enhancement of their utility in therapeutics, bioengineering strategies can be utilized to navigate cell composition and their three-dimensional (3D) organization. However, revolutionary advancements in organoid research have led to multiple in-vivo clinical applications with promising outcomes.

Keywords: Organoids, organoid transplantation, adult stem cells, pluripotent stem cells, induced pluripotent stem cells

1. Introduction

Organoids are three-dimensional and miniature organs produced in vitro that mimic key structural and functional aspects of real organs.[1] Moreover, they are developed via multiple regulated processes. Different stem cell types, such as induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), and adult stem cells (ASCs), can be utilized to create organoids. iPSCS are created by reprogramming adult somatic cells, such as skin fibroblasts, back to a pluripotent state by introducing specific transcription factors.[2] Once created, iPSCs can be directed to differentiate into specific cell lineages by exposure to defined culture conditions and signaling molecules. Likewise, the inner cell mass of blastocyst-stage embryos gives rise to ESCs, which have the capacity to differentiate into any type of cells.[3] Similar, to iPSCs, ESCs can be developed in particular ways that encourage organoid self-organization. In contrast, ASCs are found in specific tissues and play a crucial role in maintaining and repairing those tissues.[4] ASCs may divide and create organoids that preserve the properties and capabilities of their original tissue by being isolated from organs like the liver or intestine, and cultured in a supportive three-dimensional matrix containing vital growth factors.[5] In the process of producing organoids utilizing this method, ESCs or iPSCs– which are collectively known as pluripotent stem cells (PSCs) – are the primordial cells which are used to grow these

organoids. The cells are cultured within a medium to initiate aggregation and promote directed differentiation. The cell clusters are then placed within a matrix to provide structural support for the cells to form a structure similar to that of endogenous tissues which develop from various germ layers of the human body (the endoderm, mesoderm and ectoderm) by interpolating various organoid differentiation protocols which generate different models for different organs.[6]

The 3D culture mediums, such as Matrigel, mimics the extracellular matrix and offers structural support, enabling appropriate spatial arrangement. Proceeding with differentiation, which involves exposing the cells to particular growth factors and signaling molecules that direct their development into the desired cell types and promote the self-organization of organoid structures. In the last stages of maturity and maintenance, the organoids are cultivated in ideal conditions with a constant supply of vital nutrients, allowing for their development and operation, making them useful models for researching human development, disease processes, and applications of regenerative medicine.[7]

Adult stem cells (ASCs), when used to produce ASC-derived organoids, do not require the step of aggregation to differentiate into a specific cell type as they are grown from tissue resident stem cells. The cells are extracted from the organ tissue and dissociated into cells from which they are placed within a medium to initiate organoid formation by providing growth factors optimal for their growth and development and to optimize cell activity to produce specific organoid models such as intestines, stomach, pancreas and taste buds.[6] Major stem cells utilized for the derivation of each organoid are summarized in Table 1.

Within the ex vivo environment, organoids emphasize heterogeneity and can undergo poor morphogenesis during self-assembly, along with the lack of stromal, vascular and immunological components. Thus, the further development of organoids will progress by the deep intellect of human organogenesis as well as the way in which these cells manage their cellular and physical microenvironment. Numerous attempts were made, by tissue engineering – by reproducing mechano-chemical cues by engineered hydrogels and micro-devices – to the stem cell niche to gain high spatiotemporal control for both cell-cell and cell-matrix interactions.[1] In this review, processes and strategies of organoid development, cases of in-vivo organoid transplantation in humans and the impact of organoids in the future are discussed.

2. Retinal Pigment Epithelium Transplantation

Human trials have proved that Retinal Pigment Epithelium (RPE) cells can be transplanted from Retinal pigment epithelium of fetal, post-mortem adult, autologous, iPSC-derived and ESC-derived origin. iPSCs offer an unlimited supply of autologous cells and do not require the use of immunosuppressants, they may carry patients' own genetic vulnerabilities contributing to disease processes, however this can be avoided by the usage of ESCs.[8]

Table 1. Summary of the type stem cells utilized for the derivation of each organoid

Organoid	Stem cell type
Retinal Pigment Epithelium	Embryonic stem cell
Intestinal	Hematopoietic stem cell Intestinal stem cell
Colonic mucosal	Adult stem cell
Brain	Human pluripotent stem cell

RPE cells possess several functions essential for vision such as, adsorption of excessive light, transportation of nutrients to and from the neuroretina, protection against photooxidation, regeneration of 11 cis-retinal for the visual cycle, phagocytosis of shed photoreceptor outer segments and, constitutes the outer part of the blood-retinal barrier. Dysfunction of the RPE leads to; age-related macular degeneration (AMD), proliferative vitreoretinopathy and retinitis pigmentosa (RP).[8]

The three techniques for subretinal RPE transplantation developed are surgical placement of RPE as an intact cell sheet (with or without scaffold), injection of RPE as a cell suspension, and macular translocation.

The delivery of RPE as patch through injection is less traumatizing however, it comes along with associated complications such as subretinal hemorrhage and proliferative vitreoretinopathy. Moreover, cell clumping, poor attachment and disorganization of RPE upon injection are the drawbacks. Macular translocation is a less surgically straight-forward technique that involves rotating the retina away from a subretinal pathology to an area of healthy RPE and is complicated by cataract, retinal detachment and diplopia.[8]

In a phase 1 and 2 clinical trial, Schwartz and associates injected human ESC-derived RPE subretinally via a small 38-gauge retinotomy in 18 patients, nine with AMD and nine with Stargardt's Macular Dystrophy (SMD). The procedure resulted in improved visual acuity in the majority of patients. No ocular or systemic safety issues were recorded aside from surgery associated complications, such as vitreous inflammation, cataract and endophthalmitis.[8]

In phase 1 and 2 study of the California project to cure blindness, an engineered patch which is an ESC-derived RPE monolayer attached to a synthetic parylene substrate, was implanted in 16 patients with advanced non-neovascular AMD with a median age of 78 years. It was reported that mild to moderate subretinal hemorrhages and macular holes were the adverse events, with one patient developing ischemic colitis, possibly linked to immunosuppression.[8]

The London Project to Cure Blindness phase 1 trial, performed sub-retinal implantation of differentiated ESC-derived RPE, but the scaffold was made of polyester membrane coated with human vitronectin to one eye in each of two patients with severe exudative AMD. The procedure exhibited survival of the RPE patch and a visual acuity gain of 29 and 21 letters in the two patients over 12 months. Further preclinical safety studies did not reveal tumorigenicity or notable proliferative capacity of the ESC-derived RPE cells. Additionally, undifferentiated ESCs were not detected in the final differentiated RPE product.[8]

Although the number of patients included in the study is too low to conclude on the clinical efficiency of the RPE patch, the report provides valuable data about the surgical technique, stability of the transplant, and the safety of the ESC-derived cells. However, ESCs raise ethical concerns and are, in contrast to iPSCs, neither autologous nor unlimited in supply.[8]

3. Intestinal organoids

Intestinal organoids have been created as a cure for chronic inflammatory disorders of the gastrointestinal (GI) tract such as Inflammatory Bowel Disease (IBD) which also includes other disease such as Crohn's disease (CD), all of which causes inflammation of the mucosal layer of the both small and large intestines which causes other painful symptoms such as GI bleeding, abdominal pain, obstruction and many other complications such as cancer formation and could even lead to patient death.[9]

Previous treatments of IBDs include therapies involving administration of monoclonal antibodies and kinases which target the inflammatory cytokines (examples like Tumour Necrosis Factor a, interleukin-23 and 17) or any immune inflammatory responses. Another treatment method would be the removal of damaged areas through surgery. However, by utilising advanced treatment techniques such as

stem cell therapy and organoid transplantation, it may generate a long-term restoration of the mucosal lining and better healing.[9]

The first trial of cells used were hematopoietic stem cells (HSCs) to cure refractory CD by replacement of the patient's immune system by autologous hematopoietic stem cell transplantation (HSCT). The trial was partly successful, however only certain patients are permitted for the treatment as adverse effects were experienced in some patients during the trial.[9]

Another stem cell that is used is intestinal stem cells (ISC) for the treatment of IBD. ISCs are extracted from the lowest part of the intestinal crypt, which were then produced into a culture in vitro using innovative organoid technology as shown in Figure 1.

The ISCs showed continuous growth within a 3D-culture environment with the aid of non-ISC cells and growth factors, and the availability of an extracellular matrix which imitate true ECM and the components found within, mimicking the environment found in vivo. Organoids were developed with the above culture system with defined factors and type I collagen, which was then delivered colonoscopically into a human patient with CD in July of 2022.[10] Follow up of the trial and patients has not been disclosed yet (April 2025).

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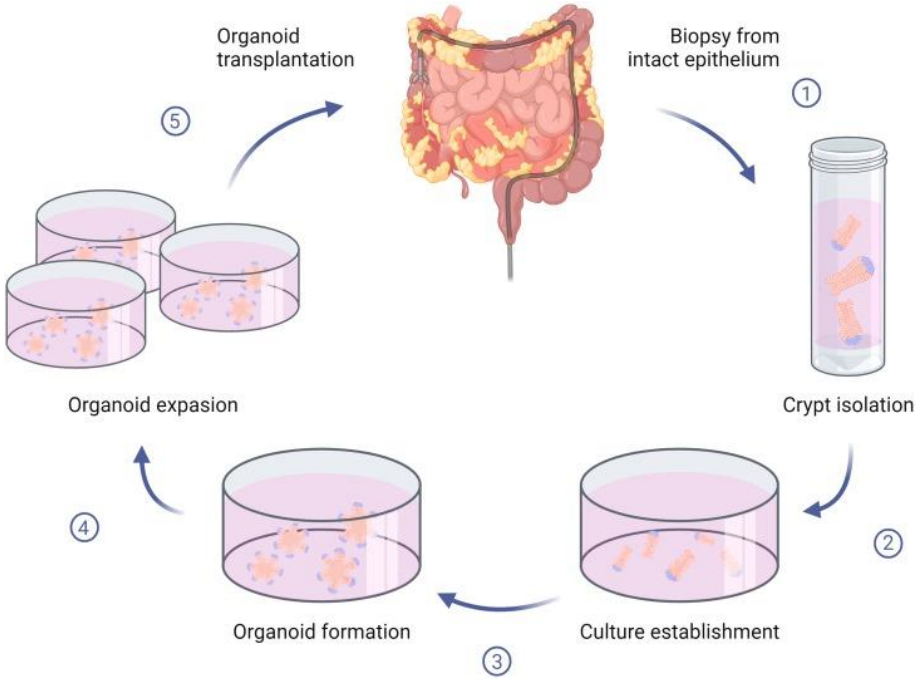

Figure 1. Intestinal organoids formation and insertion for regenerative therapy [Adapted from[11]]

4. Colonic mucosal organoids

Ulcerative Colitis (UC) is a chronic illness characterized by inflammation of the colon, prevalent among young adults, resulting in symptoms such as fatigue, bloody diarrhea and abdominal pain. It can spread through the colon after beginning in the rectum, furthermore, affecting the joints, skin and eyes.[12] Research have demonstrated that patient-derived colonic mucosal organoids can be utilized as regenerative therapy for ulcerative colitis.

The development begins by collecting a small sample of healthy colon tissue from the patient using a colonoscopy. This is a gentle, non-surgical method. The cells from the tissue are then grown in a lab for about one month. During this time, they form spherical organoids measuring approximately 0.1 to 0.2 millimeters in diameter, like miniature versions of the colon lining. Once ready, the organoids are transplanted back into the patient's colon using another colonoscopy.[13] Since the cells come from the same person, there is no risk of rejection. The method is also less invasive because it avoids major surgery.

Patient-derived colonic mucosal organoid transplantation has shown exceptional results in pre-clinical tests, where mice exhibited healing of the colon lining and improvement in symptoms. In the first human trial, the transplant patient had a better prognosis sufficient to go home the progressive day. Since the results were promising, the research team looks forward to treating up to eight patients and monitoring their progress for one year to ensure safety and efficacy of the treatment.[12]

Although this novel treatment demonstrated promising results, several challenges remain. Currently, only one patient has been treated, therefore the sample number included in the study is too low to conclude on the efficacy of colonic mucosal organoids. Furthermore, organoids developed were very small (0.1– 0.2 mm), limiting repair to small areas and making it difficult to treat widespread damage. The process also depends on using healthy tissue from the same patient, which may not be possible if there are no healthy mucosa available, moreover culturing organoids requires approximately a month, therefore making it unsuitable for urgent treatment. Long-term effects in humans are yet unknown, and there's a small risk of unexpected alterations in lab-grown cells. Since stem cells cannot be cultured alone for direct use, the method is limited to localized repair and is unsuitable for extensive or systemic disease.[13] Due to the novelty of the treatment, more time and effort is required to completely understand the long-term risks and adverse effects. As a result, more research is essential before it becomes a standard treatment.

5. Brain organoids

Brain organoids are 3D structures derived from human pluripotent stem cells (hPSCs) that recapitulate key aspects of early human brain development. These organoids self-organize into layered, functional tissues resembling specific brain regions such as the cortex, midbrain and hippocampus.[14] They are cultured under defined conditions, including extracellular matrices and bioreactors, where brain organoids offer unprecedented opportunities to study human-specific neurodevelopmental processes. Two main strategies are employed to generate brain organoids: unguided and guided approaches.[15]

Unguided methods leverage the intrinsic self-organizing properties of hPSCs, allowing them to spontaneously differentiate into a variety of brain-like structures, including forebrain, midbrain, and retinal regions. Although this method results in high cellular diversity, it often suffers from considerable inter-organoid variability. In contrast, guided methods utilize external signals such as growth factors and small molecules to direct hPSCs differentiation toward specific brain regions like the cortex or midbrain. While this approach yields more homogeneous and reproducible organoids with reduced heterogeneity, it may restrict the development of natural cytoarchitecture.[15]

Recent innovations have led to the development of "assembloids," in which region-specific organoids are fused to model inter-regional neural interactions, such as interneuron

migration and synaptic connectivity.[16] Additional advancements, including microfilament scaffolding and miniaturized bioreactors, have further enhanced the structural stability and scalability of organoid cultures. Each methodology serves distinct research goals, Unguided methods are suitable for studying whole-brain development and cellular diversity, while guided methods provide consistent models of specific brain regions. Assembloids, meanwhile, enable the study of complex neural circuits.[16]

Therapeutically, brain organoids serve as powerful platforms for modeling neurodevelopmental and neurodegenerative diseases such as microcephaly, epilepsy, Alzheimer's disease (AD), and autism spectrum disorders (ASD).[17] Patient-derived organoids support personalized medicine through individualized drug screening and genomic analysis. For example, in ASD, organoids derived from patients have revealed abnormal neurogenesis, altered synapse formation, and disrupted neural connectivity, helping to elucidate the developmental origins of the disorder. In Rett syndrome, organoids generated from iPSCs with Methyl-CpG Binding Protein 2 (*MECP2*) gene mutations show impaired neuronal maturation, reduced dendritic complexity, and gene expression abnormalities. Organoids infected with the Zika virus mimic microcephaly by exhibiting reduced proliferation of neural progenitors and smaller organoid size. For AD, brain organoids display hallmark features such as amyloid-β plaques and tau pathology, providing a model to study disease progression and test therapeutics. Midbrain organoids from Parkinson's disease (PD) patients exhibit dopaminergic neuron degeneration and mitochondrial dysfunction. Similarly, Huntington's disease (HD) organoids demonstrate mutant huntingtin protein aggregation, neuronal loss, and gene dysfunction, offering platforms for testing gene-editing strategies.[17]

A promising future direction involves the transplantation of brain organoids into living organisms. This in vivo approach aims to integrate human-derived neural tissues into host brains to repair damage, restore function, or replace degenerative cells. Studies have shown that transplanted cortical organoids can survive, vascularize, and form functional synaptic connections in rodent models. However, significant challenges persist, including poor vascularization, limited cell-type diversity (notably the absence of microglia and oligodendrocytes), and central necrosis due to hypoxia.[16] Ethical considerations, particularly those related to cognitive capacity in chimeric animals, demand stringent oversight. To address these issues, ongoing research focuses on enhancing functional integration, vascular support, and immune compatibility. The use of assembloids combining various brain regions and cell types shows particular promise in promoting neural maturation and interconnectivity.[16] While the path to clinical application of brain organoid transplantation is complex, it holds transformative potential for treating a range of neurological disorders, provided that scientific, ethical, and translational challenges are addressed with diligence and care.

6. Current Challenges in organoid transplantation

Organoid technology has rapidly advanced, yet it still faces several critical challenges that limit its effectiveness as a cell culture model. One major limitation is the lack of vascularization, which restricts nutrient and oxygen diffusion, thereby limiting organoid growth and leading to necrotic cores in thicker tissues.[7] To address this, researchers have explored the use of microfluidic devices that replicate blood flow, as well as liver sinusoidal cells that promote vascularization in liver organoids.[18] Another significant challenge is the poor reproducibility of organoid cultures due to inconsistencies in size, shape, and cellular composition, which complicates data analysis and study design.[7] Standardizing culture protocols and integrating automated tools to regulate the microenvironment have been proposed as solutions to enhance consistency[20]. Additionally, organoids often fail to fully mimic the complexity and functionality of mature organs due to limited cellular diversity, incomplete maturation, and insufficient interaction with surrounding tissues.[19] To

improve this, co-culturing organoids with supportive cell types, introducing mechanical stimuli, and utilizing biomaterials that provide biochemical and mechanical signals have been recommended.[7] Ethical and safety concerns, particularly regarding ESC derived organoids and immune rejection, also present significant barriers.[8] Efforts to address these issues include gene editing techniques, such as Clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9 (CRISPR-Cas9), to modify Human Leukocyte Antigen (HLA) genes in allogeneic organoids for immune compatibility, as well as refining surgical techniques and microsurgical tools for safer transplantation procedures.[21] Furthermore, the use of Matrigel, derived from mouse sarcoma, limits the generation of human-transplantable organoids, necessitating the development of synthetic biomimetic scaffolds as an alternative.[4] Although significant progress has been made in overcoming these challenges, further research and clinical trials are essential to establish the long-term efficacy, safety, and scalability of organoid-based therapies. Such advancements made towards the 3D generation of organoids, have paved the way towards clinical applications.

7. Conclusion

The ability to create miniature, functional models of organs using stem cells is not simply a remarkable scientific achievement, it has proven to be a novel approach for the next generation of personalized and regenerative medicine. Furthermore, organoids would be crucial for personalized treatment in the future, as patient derived tissues enable toxicity screening and medication testing that is specific to each patient's genetic background. It is promising that organoids would be able to successfully replace damaged tissues or organs as bioengineering techniques advance. It is also evident that they possess the potential to become feasible for transplantation upon overcoming obstacles such as vascularization and immunological compatibility. Moreover, lab-grown tissue may be used to treat conditions such as intestinal disorders or liver failure that presently require donor organs, avoiding long transplant waiting periods. The use of organoids in disease modeling also creates new opportunities for research into illnesses that are otherwise hard to reproduce in animals and to also provide a more ethical approach for research in medicine and disorders, particularly in genetic or neurological disorders. In addition, they aid in providing insights on preventing diseases before symptoms appear, accelerating treatment discoveries, bringing upon a great impact in the future of medicine.

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