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Review Article

Organoids As Promising Tools In Therapy

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ABSTRACT

The historical reliance of biological research on the use of animal models has sometimes made it challenging to address questions that are specific to the understanding of human biology and disease. But with the advent of human organoids - which are stem cell-derived 3D culture systems, it is now possible to re-create the architecture and physiology of human organs in remarkable detail. Human organoids provide unique opportunities for the study of human disease and complement animal models. It is been used to study infectious diseases, genetic disorders and cancers through the genetic engineering of human stem cells, as well as directly when organoids are generated from patient biopsy samples. This review discusses the types and various applications of human organoids as models and outlines the challenges that have to be overcome for organoids to be able to substantially reduce the need for animal experiments.

INTRODUCTION

Organoids are tiny, self-organized three-dimensional tissue cultures that are derived from stem cells. Such cultures can be crafted to replicate much of the complexity of an organ, or to express selected aspects of it like producing only certain types of cells. They grow from stem cell- cells that can divide indefinitely and produce different types of cells as part of their progeny. There are potentially as many types of organoids as there are different tissues and organs in the body. To date, researchers have been able to produce organoids that resemble the brain, kidney, lung, intestine, stomach, and liver, and many more are on the way[1].

Drug-induced adverse drug reactions are one of the leading causes for the discontinuation of drug development projects and the withdrawal of drugs from the market. It is, thus, essential to minimize adverse reactions to improve patient safety. For pharmaceutical companies, the decision to discontinue drug development due to adverse reactions at an early stage leads to a reduction in development costs and human resources. Therefore, studies focused on accurately predicting potential drug-induced adverse effects

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in humans in the early stages of drug development are important. Toxicity studies have previously relied on animals, but species differences in anatomy, function, and morphology in various tissues have hampered the accurate understanding of the mechanisms of toxicity and the acquisition of accurate predictions regarding toxicity in humans. Additionally, animal studies are expensive and time-consuming, which can limit the number of substances tested. Therefore, in vitro assays using human cells are required to overcome these problems and to align with the concept of the 3Rs (replacement, reduction, and refinement) [2].

Model organisms

Caenorhabditis elegans

Caenorhabditis elegans is a species of nematode worm and is frequently chosen as a model organism to study human diseases. *C elegans* is a species of nematode worm, and the adults are approximately 1mm in length with 959 somatic cells. Its transparent body consists of three layers; an epidermal layer, an intestinal layer, and a muscle layer. In 1963, Sydney Brenner proposed using *C. elegans* as a model organism for the investigation primarily of neural development in animals. It has been used as a model organism to study molecular mechanisms in metabolic diseases. The transparency of *C. elegans* facilitates the study of cellular differentiation and other developmental processes in the intact organism. Maintenance is easy when compared to other multicellular model organisms. A few hundred nematodes can be kept on a single agar plate and suitable growth medium. Brenner described the use of a mutant of *E. coli* – OP50. OP50 is a uracil-requiring organism and its deficiency in the plate prevents the overgrowth of bacteria which would obscure the worms. The use of OP50 does not demand any major laboratory safety measures, since it is non-pathogenic and easily grown in Luria-Bertani (LB) media overnight [3,4].

Fig: 1 ***Caenorhabditis elegans***

Drosophila melanogaster

The fruit fly, *Drosophila melanogaster* is used as a model organism to study disciplines ranging from fundamental genetics to the development of tissues and organs. Drosophila genome is 60% homologous to that of humans, less redundant, and about 75% of the genes responsible for human diseases have homologs in flies. These features, together with a brief generation time, low maintenance costs, and the availability of powerful genetic tools, allow the fruit fly to be eligible to study complex pathways relevant in biomedical research, including cancer [5]. Most of the signaling pathways controlling cell growth and invasion in mammals have a conserved function in flies allowing their modulation into models that mimic tumor's biology in a simple model organism like Drosophila. The combination of genetic screens with the availability of powerful recombination techniques enabled also a rapid characterization of the primary function of conserved oncogenes and of tumor suppressor genes in a whole animal. In addition, recent studies using Drosophila imaginal discs explored the mechanisms that govern growth in epithelial tumors and their interaction with the local TME and stromal cells, including some steps in the recruitment of the immune cells (macrophages) to the tumor mass [6,7].

Fig: 2 *Drosophila melanogaster*

Zebrafish

The zebrafish model organism has been used to elucidate the genetic and cellular mechanisms related to development since the embryo forms and grows externally following fertilization. This provides insight into the genetic control of developmental processes in humans because their genomes are similar [8,9]. The zebrafish is a promising model for studying age-related changes in cognition and perception. Zebrafish exhibit characteristics that are similar to humans, as well as other mammals, including the fact that these animals age gradually, and they demonstrate aging-related changes across both cognitive and neurobiological spectrums. It clear that both genetic and non-genetic interventions can be applied to alter the course of the aging process and provide potential drug targets that could be manipulated to ameliorate age-related cognitive declines. Therefore, this model will help researchers elucidate the biological mechanisms that underlie aging-related cognitive declines[10].

Fig: 3 Zebrafish

Types of organoids

A multitude of organ structures have been recapitulated using organoids. This section aims to outline the state of the field as of now through providing an abridged list of the organoids that have been successfully created, along with a brief outline based on the most recent literature for each organoid, and examples of how it has been utilized in research[11].

Cerebral organoids

Cerebral organoid, or brain organoid, describes an artificially grown, in vitro, miniature organ resembling the brain. Cerebral organoids are created by culturing pluripotent stem cells in a three-dimensional rotational bioreactor, and they develop over a course of months. The purpose of creating an in vitro neurological model is to study these diseases in a more simple and variable space. Cerebral organoids are synthesized tissues that contain several types of nerve cells and have anatomical features that recapitulate regions of the cortex observed in brains. Cerebral organoids are most similar to layers of neurons called the cortex and choroid plexus. In some cases, structures similar to the retina, meninges and hippocampus can form[12,13].

Gut organoid

Gut organoids refer to organoids that recapitulate structures of the gastrointestinal tract. The gastrointestinal tract arises from the endoderm, which during development forms a tube that can be divided in three distinct regions, which give rise to, along with other organs, the following sections of the gastrointestinal tract: The Foregut gives rise to the oral cavity and the stomach. The Midgut gives rise to the small intestines and the ascending colon. The Hindgut gives rise to the rectum and the rest of the colon[14].

Intestinal organoid

Intestinal organoids are a three-dimensional in vitro model of the human intestinal epithelium that allow for robust, patient specific in vitro research

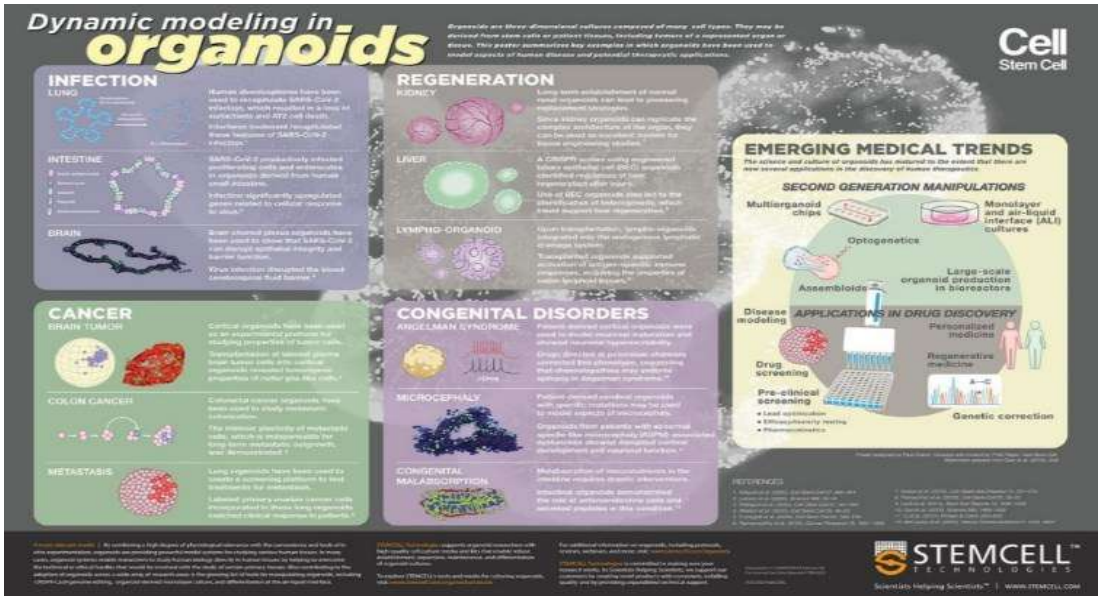
of the development and properties of the intestinal epithelium[15]. The prevalence of Inflammatory Bowel Diseases (IBD) is rapidly increasing across both developed and developing countries (1). IBD, such as Crohn's disease (CD) and Ulcerative colitis (UC), affects up to 0.5% of people in the Western world (1). Due to a lack of patient specificity and knowledge of disease mechanisms, successful treatment of these diseases remains difficult. Frontline IBD treatments have limited efficacy in large groups of patients[16]. For example, Infliximab, a biologic anti-tumor necrosis factor (TNF) antibody treatment, is only effective in 60–87% of patients, 23–46% of whom become secondary non-responders within 5 years (2). Mechanisms of IBD are yet to be elucidated and are difficult to pinpoint in individual patients[17].

Lung organoids

The "lung organoids" are one of many kinds of organoids. Like other organoids, the lung organoids can derive from stem cells or organ-specific progenitors through a self-organisation process. The cultured process of lung organoids in vitro is strikingly different compared with that of a traditional cell culture. The lung organoids can simulate the developmental process of the lung, as well as recapitulate the three-dimensional (3D) organisational structure (such as alveolars, airways, and lung buds) and function of the lung *invitro*. They are used widely to study pulmonary diseases[18].

Brain organoids

Brain organoids offer an in vitro approach to study aspects of human brain development and disease. Diverse brain organoid techniques offer bioassays to investigate new phenotypes associated with human brain disorders that are difficult to study in monolayer cultures. Brain organoids have been particularly useful to study phenomena and diseases associated with neural progenitor morphology, survival, proliferation, and differentiation[19,20].



Methods for *invitro* organoid formation

Generally, there are three approaches to establish 3D organoids *invitro*. The first method is organoid generation on the ECMs (e.g: EHS, collagen, etc.) in plates, known as 3D simple culture[21]. This method, however, has some drawbacks, including (a) lack of nutrient and gaseous exchange and waste removal, (b) lack of reproducibility and uniformity of organoids that makes it an improper platform for large drug screening or high-throughout evaluating. The second method is using spinning bioreactors to generate organoids from EBs. Although bioreactors enhance nutrient supply, they are usually restricted by the size of

organoid. This method is often utilized for retinal and cerebral organoids generation. As an example, Lancaster and colleagues modelled some aspects of microcephaly in PSCs-patient-derived cerebral organoids. In order to develop organoids, they utilized spinning bioreactors for cells floating in suspension to induce iPSCs and form EBs. The third method is air-liquid interface (ALI), by which, the top layer of cells is exposed to air, while the basal surface is in contact with the liquid medium (i.e, culture media in organoids)[22].This method is mainly used to generate kidney and intestinal organoids.

Microfluidic as the major device in ALI technology can control and optimize nutrient, gases, temperature, pH and waste removal. Indeed, regulating the key parameters, including cell-cell and cell-ECM interaction, fluid-flow exchange, tissue patterns, and electrical stimulation, can greatly reduce batch-to-batch variations and increase the tissue similarities. Moreover, sensors and actuators can be set in the microfluidic devices to enable precise monitoring and control[23]. Thereby, the microfluidic technology can facilitate the complex nature of 3D organoid culture through organoid-on-a-chip and organ-on-a-chip models. Through microfluidic organoids-on-a-chip, a variety of organ models, such as liver, kidney, heart, lung, and neural networks, has been successfully mimicked. Notably, this technology allows the integration of multiple tissues to resemble the multiple organs for wide drug screening and precise predictions. Recently, multiorgan chips indicated the promising results to mimic the physiological and biological complexity of the human body and establish the interactions between different organs. As an interesting model, Zhang et al generated modular multiorganoids on a chip with the combination of non-invasive physical, biochemical, and optical sensors for online organoid monitoring. They demonstrated the successful generation of heart and liver organoids on a chip platform and confirmed the ability of in situ biosensors. They further monitored the micro environmental parameters (e.g, pH, O_2, temperature) and measured the related biomarkers by electrochemical immune biosensors. In another study, a four-organ-on-a-chip, including skin, liver, intestine, and kidney, was generated. They indicated the modules of intestine, skin, and kidney grown in 2D structures, while liver buds represented 3D structure. In their microchip, they utilized the fluid-to-tissue ratio as a physiological parameter and reliable tool for future distribution, absorption, and metabolism investigations[24].

APPLICATION OF HUMAN ORGANOID

Organoid in biomedical research

Since organoids are derived from human stem cells or induced pluripotent stem cells, they provide the genetic and physiological similarity required for the enhanced modelling of human development and disease, in comparison to current animal models.

Organoids are currently being employed in biomedical research to:

1. Examine organ development and tissue morphogenesis.
2. Model diseases.
3. Test drug sensitivity and toxicity.
4. Potentially form complex tissues for transplantation.

Study of infectious disease

Organoids are particularly well suited as a model for the study of infectious disease, given that all cell types from an organ system can be generated *invitro*, providing insight into the interactions between pathogens, and specific cells types that are required for host infection. For example, *C. difficile* infection is quite common among hospitalized patients, the complications of which range from intractable diarrhea and pain to septic shock, toxic megacolon, and death. After infecting human intestinal organoids

with *C. difficile*, researchers found that the secreted bacterial toxin inhibited the barrier function of intestinal cells, and diminished expression of a Na^+/H^+ exchanger (NHE3), both contributing to the pathogenicity of *C. difficile*.

Examples exist that leverage organoids to model infection with *Helicobacter pylori, Cryptosporidium parvum, Salmonella enterica, Toxoplasma gondii,* Rotavirus, Norovirus, influenza, and Zika virus. Qian *et.al.,* used induced human PSCs to generate forebrain-specific organoids that recapitulated key features of cerebral development. These brain organoids were later infected with strains of Zika virus, where increased neuronal cell death and decreased neuronal layer thickness were noted, resembling microcephaly[25].

Organoid and regenerative medicine

Organoids may be applied to the field of regenerative medicine. By combining organoid systems with biofabrication strategies, complex tissue or organ functions can be replicated. Current advances in developing intestinal organoids are providing the potential for organoid-based therapeutic treatments for patients with short bowel syndrome and human inflammatory bowel disease[26].

Organoid and personalized medicine

Personalized medicine promises to provide patients with the most suitable treatment that is specific to them. Organoid systems are an important tool in developing personalized medicine because they are derived from a single patient biopsy meaning that the culture will display genetic similarity. Tests of drug efficacy can therefore be performed on organoid systems rather than the lengthy trial and error testing of treatments prescribed to the patient. The methodology has already proven successful in identifying individual treatment outcomes for cystic fibrosis. The mutated cystic fibrosis transmembrane conductance regulator (CFTR) affects the production of the CFTR protein which causes cystic fibrosis.

Organoid and cancer research

Cancer research has been limited due to the lack of in vitro models that can accurately replicate the physiology of the original tumor. In 2017, organoids of primary liver cancers were propagated preserving the physiological architecture and gene expression of the original tumor. The results of *in-vivo* transplantation in mice found that the long term in vitro expansion of the cancer-based organoids still preserved the parent tumor histology, by the secondary tissues derived from the grafted tumors exhibiting similar omosome counts and identical morphology to the parental line.

Moreover, the cancer-based organoids were utilized in a successful array for drug sensitivity testing. The results mean that organoid systems which maintain the histology and gene expression of the original tumor can be employed in predicting drug sensitivity and specific[27].

Conclusion

Organoids are three-dimensional assemblies that contain multiple cell types, arranged similarly to the cells in a specific tissue, at least at the micro-scale; mini-organs add to this micro-realism a realistic macro-scale anatomy as well. Researchers have been producing organoids for at least 60 years, initially to explore basic mechanisms of development, but more recently as tools for medical research. Organoids made from human cells are particularly valuable for preclinical studies, because they avoid the need to extrapolate results from one species to another. This review outlines the types and various applications of organoids.

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