Proceedings of the 4th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/40/20241231

Review on the development of the organoids

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Abstract. Organoids, a novel field, were first experimentally supported in 2007 and began to be engineered around 2020. Organoids can be developed from stem cells or organ progenitor cells through the process of differentiation, or they can be created by changing the alignment of pluripotent stem cells throughout the process of cell differentiation. Organoids are exploited for the purpose of scientific research in the areas of human development, illness, screening of medications, and gene therapy. The study utilized a literature review method to examine the evolution of organoid production, new application, and technological advancements. Organoids have advanced from basic tissue engineering organs to being used for modeling diseases and providing more therapeutic options. Their progress is noteworthy and has the potential to be a significant approach in biological medicine research in the future.

Keywords: Organoids, history of organoid, source organ

1. Introduction

Organoids are small tissue structures that replicate real organs, capable of being grown in a laboratory setting and displaying comparable structures and functions. Organoids can be distinguished from stem cells or organ progenitor cells through differentiation, or they can be created by manipulating the alignment of pluripotent stem cells. Organoids are utilized for investigating human development, disease, medication screening, and gene therapy. Organoid research is an innovative area within life sciences with significant applications and possibilities. Organoids are 3D in vitro cell culture systems that mimic the intricate spatial structure and cell-cell interactions of a specific tissue or organ. Organoids consist of diverse cell types that create operational "micro-organs" mirroring the physiological reactions and traits of the original tissue or organ. Organoids offer a more accurate representation of organogenesis and physiopathology compared to the conventional 2D cell culture paradigm, making them valuable for basic research and therapeutic purposes. An organoid is defined by the presence of one or more cell types identical to those of the source organ, unique functions specific to the source organ, and a cell arrangement resembling that of the source organ.

This paper provides an overview and analysis of the historical evolution and technological advancements of organoids through literature review and analysis. It partially offers references for this field.

DOI: 10.54254/2753-8818/40/20241231

2. Literature review

2.1. Early Basic Research

Organoid research is an emerging discipline whose origins date back to 2007, when Hans Clevers' lab discovered Lgr5+ stem cells in the small intestine and colon. Two years later, they used a single murine LGR5+ intestinal stem cell to produce an intestinal organoid with intestinal crypt-villus structure in vitro. This was the first time that a miniature model of a complex organ in the human body was successfully produced [1]. In 2011, human intestinal-like organs were also successfully fabricated, either differentiated from human pluripotent stem cells or from primary adult stem cells. In the same year, retinal-like organs were also successfully cultivated for the first time, differentiated from murine embryonic stem cells [2]. In 2012, human pluripotent stem cells were also used to create retinal-like organs [3]. Organoid research spurt from 2013 to 2015, brain-like organs, liver, kidney, pancreas, and other external organs such as prostate and lung were also successfully cultivated [4-7]. By 2015, other complex tissue-like organs such as mammary glands, fallopian tubes, and hippocampus were also successfully cultivated [8-10]. In 2020, snake venom gland-like organs were successfully cultivated, which was the first time that non-human animal-like organs were created [11].

2.2. Recent Research

In recent research, the discipline of organoids has begun to gradually enter the application phase. The use of organoids to construct models to study corresponding diseases and the use of organoids to repair organ damage have become the focus of research.

In February 2021, bile duct organoids were used to repair damaged human livers, which was the first time that organoids were applied to the repair of human organs [12]. In August 2021, brain organoids containing optic cups were successfully cultivated, which was the first time that primitive sensory structures were reproduced in brain organoids [13]. In November 2021, trophoblast organoids were used to study female early pregnancy and pregnancy complications, which was the first time that organoids were used to simulate the interaction between mother and fetus [14]. In the same month, brain organoid section models were used to study the molecular pathology of amyotrophic lateral sclerosis combined with frontotemporal lobe dementia, which was the first time that organoids were utilized to simulate mature cerebral cortical structures [15]. In April 2022, a comprehensive analysis of pancreatic cancer organoids revealed chromatin accessibility features related to drug sensitivity, which was the first time that organoids were utilized to explore the chromatin accessibility of pancreatic cancer and gene regulatory networks [16].

On February 23, 2023, Hans Clevers' team researched and established new human fatty liver organoid models and used these organoid models to elucidate drug responses, as well as established a CRISPR screening platform based on the organoids and successfully screened for a potential new target for the treatment of fatty liver, the FADS2 gene. These organoid models and screening platforms will help test and develop new drugs for the treatment of fatty liver and contribute to a better understanding of disease biology [17].

On 2023-02-27, researchers from the Guangdong Lung Cancer Research Institute and their collaborators used patient-derived organoids to predict tumor efficacy in locally advanced or metastatic lung cancer, and the results of the study showed that lung cancer organoids predicted clinical efficacy with an overall accuracy of up to 83.3%, which is the largest cohort of published studies of lung cancer organoids worldwide [18].

Researchers from Cincinnati Children's Hospital and other institutions have created an improved model for studying human gastrointestinal diseases. Their research led to the development of a new type of complex intestinal organoids that contain essential elements of the immune system. This is the first model of its kind to include a functional immune system along with other vital organs like the heart, liver, and stomach, developed by scientists to date [19].

Zhicheng Shao's team from Fudan University developed a method to induce astrocytes into neural organoids (called Op53-CSBRY), and the organoids generated through the Op53-CSBRY method can

DOI: 10.54254/2753-8818/40/20241231

form spinal cord organoids with dorsal and ventral spinal cord neuronal functions after the addition of bFGF, SAG, and BMP to activate the signaling pathways related to spinal cord development. After transplantation of the spinal cord organoids to mice with spinal cord injury, the organoids survived and differentiated into spinal cord neurons and formed synapses with host neurons, improving motor function in mice. This study makes a big step forward to the idea of direct reprogramming of human astrocytes into neural-like organs in vivo to repair neurological injuries [20].

3. Technology Development

3.1. Preparation Platforms

Cell self-aggregation platform, penetrable chamber platform and microfluidic control platform are mainly included in the preparation platform of endosome. Cell self-aggregation platforms mainly include bioreactors, microdroplet method, low-attachment plates and magnetic levitation method, they simulate the environment. The dissociated cells are induced to self-assemble into tissue-like structures. The penetrable chamber platform employs gas-liquid interface technology and is commonly utilized for simulating respiratory epithelial cells, in vitro skin models, and similar applications [21-23]. Finally, there is the microfluidic preparation platform. Based on a microfluidic print head, the device is able to adjust the concentration of printed cells in real time. The device allows bioprinting at high cell concentrations. The authors of the paper demonstrated that this method can be used to print bladder-like organisms [24].

3.2. Simulated extracellular matrix

Organoids are three-dimensional cell aggregates cultured in vitro that mimic the structure and function of real organs. Organoid culture requires a suitable matrix gel, a hydrogel material that provides an extracellular matrix (ECM). The ECM is a complex network of multiple proteins and polysaccharides that support cell attachment, migration, proliferation, differentiation and signaling. The composition and properties of ECM vary in different organs and tissues, so the selection of a suitable matrix gel is important for organoid formation and function [25].

Matrigel Matrix Gel. The most widely used matrigel matrix gel is a basement membrane matrix derived from EHS mouse tumors enriched in extracellular matrix proteins, which contains approximately 60% laminin, 30% IV collagen, and 8% nestin. matrigel matrix gel also contains basement membrane glycans, TGF-β, epidermal growth factor, insulin-like growth factor, tissue plasminogen, and other growth factors [26]. Matrigel matrix gel is a complex mixture of naturally occurring bioactivities and biochemical characteristics that mimic the extracellular environment in vivo and are suitable for a variety of in vitro 2D and 3D cell culture applications, such as cell adhesion, proliferation, differentiation, migration, invasion, angiogenesis, neurobiology, stem cells, and organoids [27].

Matrigel matrix gel is the most traditional matrix gel for organoid culture, but there are some limitations and challenges. Researchers are developing and exploring other types of matrix gels, such as animal-derived matrix gels, tissue decellularized matrix gels, plant-derived matrix gels, and synthetic matrix gels, which have different characteristics and advantages and can be selected and optimized for different organoids and applications.

Emerging new matrix gel. Hydrogel matrix prepared from decellularized porcine small intestine can better support the growth and expansion of endodermal organoids such as stomach, intestines, liver, pancreas, and other organoids compared to matrigel.

Porcine small intestine sourced hydrogel used to culture intestinal-like organs is also an animal source, but the porcine small intestine material is easy to obtain, has a high yield, and is not a tumor source, which naturally has a great advantage. It is also possible to achieve GMP production specifications [28]. The hydrogel prepared from decellularized porcine brain extracellular matrix (B-ECM (ecellularized porcine brain)) was used as a matrix gel for human embryonic stem cell (hESC)-

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derived brain organs, which has the biochemical characteristics of natural brain tissues and meets the criteria for clinical application [29].

Type I collagen matrix gel: It has a simpler composition than matrigel matrix gel and its physical properties are easier to manipulate. Experiments have been conducted using human induced pluripotent stem cell (iPSC)-derived neural precursor cells (NPCs) to form and grow into neural-like organs in synthetic type I collagen matrix gels [30]. This type of hydrogel also supports the survival and growth of intestinal organoids.

3.3. Growth Factors in Organoids

Growth factors are an important part of organoids as they can influence organoid formation, development and function. Different organoids require different growth factors, depending on their cellular origin, tissue type and signaling pathways. Growth factors mainly include growth factors that support stem cell proliferation and differentiation as well as small molecule inhibitors.

Wnt Family: Wnt is a family of signaling molecules involved in a variety of cellular processes including cell polarity, migration and proliferation. In organoid culture, the Wnt signaling pathway plays a key role as it triggers stem cell self-renewal and differentiation. For example, Wnt-3a promotes the development and growth of organoids including the small intestine, liver, kidney, pancreas, prostate, and mammary gland.

spondin family: R-spondins are a group of secreted proteins that work synergistically with the Wnt signaling pathway to enhance Wnt signaling by binding to the Lgr5 receptor, a hallmark of stem cells, indicating that the cells have the capacity for self-renewal and development. Lgr5 is a hallmark of stem cells, indicating the ability of the cell to self-renew and develop into organoids. For example, R-spondin-1 promotes the development and growth of organoids such as the small intestine, liver, kidney, pancreas, prostate and mammary glands.

Noggin: Noggin is a secreted protein that inhibits bone morphogenetic proteins (BMPs), which play a role in the regulation of cellular differentiation, proliferation and apoptosis.The BMP signaling pathway is commonly inhibited in organoid cultures because it prevents stem cell proliferation. BMP signaling pathway is often inhibitory in organoid cultures because it prevents stem cell proliferation and differentiation. For example, Noggin promotes the development and growth of organoids such as small intestine, stomach, liver, lung, pancreas, prostate, mammary gland, and brain.

EGF family: EGF is a group of signaling molecules that are involved in cell proliferation, differentiation, migration, and survival, and they activate the downstream signaling pathway by binding to the EGF receptor.The EGF signaling pathway has a positive role in organoid cultures because it promotes cell proliferation and differentiation. The EGF signaling pathway plays an active role in organoid culture because it promotes cell proliferation and differentiation. For example, EGF promotes the development and growth of organoids such as the small intestine, stomach, liver, lung, pancreas, prostate, breast, and brain.

In addition to the previously mentioned growth factors, organoid media incorporate a variety of small molecule inhibitors, such as CHIR99021, Y-27632, A83-01, and SB431542, which are critical for the maintenance of stem cell self-renewal and proliferation by specifically interfering with signaling pathways, blocking or activating. These inhibitors have been widely used in multiple organoid culture media, where they help prevent cell death and promote organoid formation [31].

The proportion and use of growth factors in organoid culture depend on different organoid types, cell sources, culture substrates, and experimental purposes. In general, the ratios and methods of growth factor usage need to be experimentally optimized for optimal organoid formation and function.

4. Conclusion

This study discusses the evolution of organoids from construction to application, highlighting early construction findings and recent application outcomes. The history of organoid research is brief, however the outcomes are abundant, making it unfeasible to document all of them in one study. The future of the discipline has great promise and also challenges. Organoids will be utilized in several

applications including drug testing, organ transplantation simulation, organ regeneration, organ mechanism research, and medical therapy in the current and upcoming years. As a nascent technology, this innovation encounters obstacles. Many existing organoids lack vascular architecture and functional systematization; in the current industry, process control is not standardized. These investigations lack consistency and statistical significance, hindering their translation into clinical research. Matrigel, the current matrix gel in the industry, holds a monopoly leading to expensive costs. The developing matrix gel is not yet fully developed, posing challenges for future industrial mass production. Overall, the industry is currently experiencing a period of rapid growth. It is both highly promising and demanding. It is likely to progress towards actual organs.

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