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Challenges in Engineering Functional Organoids for Research

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ABSTRACT

Organoids have emerged as transformative tools for studying human development, disease modeling, and drug discovery due to their ability to mimic in vivo tissue architecture and functionality. However, despite their promise, significant challenges persist in engineering organoids with precise functionality and reproducibility. This review discusses the limitations of current organoid systems, including their inability to fully replicate native tissue environments and the variability in outcomes. Key engineering approaches, such as biomaterial-based scaffolds, cell source optimization, and differentiation protocols, are evaluated for their role in enhancing organoid maturation and functionality. Furthermore, the importance of functional characterization and its standardization is highlighted to ensure the physiological relevance of organoid-based models. The paper concludes with an exploration of the diverse applications of organoids in disease modeling, personalized medicine, and regenerative therapy, emphasizing the need for multidisciplinary collaboration to address the remaining challenges and fully realize the potential of organoid technology in biomedical research.

Keywords: Organoids, 3D tissue models, biomaterials, scaffolds, functional characterization, cell differentiation.

INTRODUCTION

In recent years, there have been important advances in the field of organoids that have cleared the path for studying human development, disease modeling, and drug testing. As promising as they are, these organoids come with certain limitations and face huge challenges that must be overcome to provide better strategies for biomedical research. The bioengineered organoids lack essential features that are typical of the in vivo tissues they are supposed to mimic. If we are to study a human entity successfully, it is essential to develop platform strategies that involve more resemblance to the functioning of humans than lesser entities like mice and other small animals. To fully engineer reproducible, safe, and scalable organoids, which are very useful for further studying drug triggers, and basic biology pathways, and developing new remedies for a range of immune-inflammatory diseases, is the need of the hour [1, 2]. There has to be wide-ranging collaboration among bioengineers, developmental logicians, physicists, material scientists, and others, as the issue of organoid engineering is vast and complex. The ultimate goal of this paper is to address some limitations and to improve the research of bioengineered organoids. Essential tools are those for growing human organoids and tissues in vitro: they can maintain up to 90% of the properties active for their in vivo sources for a year or more and more closely resemble living people. Organs-on-a-chip platforms feature human biologicals and offer the ability to create or restore the growth of a plethora of promising devices. Platform systems, including organoids for models, allow non-invasive discovery, personalized screening, and proliferative biology for disease study and treatment. Organs-on-chips are versatile biological components that mimic the passage through multiple tissue representations [3, 4].

Overview Of Organoids

Organoids are three-dimensional (3D) structures that replicate the architectural characteristics and functions of their in vivo counterparts. Organoids were initially developed using pluripotent or multipotent stem cells that can self-organize and differentiate into diverse cell types, yielding a heterogeneous, self-renewing pattern. A wide range of organoids have been produced for various applications. For example, the intestinal organoid was created to mimic crypt-villus structures and to explore stem cell responses to damage or pathogens in the gastrointestinal tract. Liver organoids have also been used to model liver development from the fetal to the adult stage and to serve as liver disease models. Finally, to help researchers gain more insight into neurological diseases, brain organoids were made as models for brain development and neurological diseases, and neural and retina organoids for nervous system malfunction [5, 6]. Biologically, the definition of organoid bridges the gap between conventional two-dimensional (2D) tissue cultures and in vivo systems because of the following respective features: organoid 3D structure for in vivo organ environments and local microenvironments with similar functionality to that of in vivo organ tissues. Over the past decade, organoids have shown great potential in identifying drugs and genetic alterations that disrupt normal organ development and function in vitro. However, there remains significant variation in organoid maturation, encompassing a wide variety of controlled and non-preferential consequences. Additionally, it should be mentioned that organoids can be easily injured or distressed under the stress of multiple cells' self-assembly. Cell death in 3D cultures is frequently caused by nutritional deficiencies at specific spatial points in organoids that cannot be reached efficiently by the culture medium. To address this, this review aims to provide some innovative and various engineered strategies for organoid generation [7, 8].

Definition and Types

Organoids by definition are self-organized and self-assembled in vitro cellular clusters that mimic their in vivo tissue of origin, and thus they are a viable step between 2D in vitro cell culture and in vivo animal models. The cellular composition and the structural complexity of the organoid can vary widely between different tissues of origin. Based on the tissue of origin, organoids can be categorized into three subtypes: 1) Ectodermal organoids, including tissues such as the brain, in the form of cortical spheroids, retina, gut-brain, etc.; 2) Mesodermal organoids, including heart, bone, etc.; and 3) Endodermal organoids, such as stomach, liver, pancreas, lung, intestine, kidney, etc. Devising strategies that can help in the generation of functional organoids that are more representative of their adult tissues could have very significant implications in the fields of drug development, regenerative medicine, personalized medicine, and tissue transplantation [9,10]. While generating organoids, researchers need to understand the tissue-specific requirements and differences to develop strategies to engineer them according to the desired application. Additionally, there are several issues and challenges associated with generating organoids for research; the majority of organoids today still do not perfectly mimic their in vivo tissue of origin, and these functional differences are further exacerbated in long-term cultures. Organoids can provide an unlimited cell source for personalized drug testing and a potential source for autologous tissue engineering. However, potential variability in organoids from patient to patient and different organoid batches will pose challenges in large-scale cell and tissue engineering. It will be important to address scalability in the future for organoid applications [6, 11].

Engineering Approaches

A logical starting point for engineering functional organoids is to ensure their growth in a suitable environment that mimics the in vivo context. This has mainly been achieved using a variety of biomaterial approaches. Scaffolds can provide support for self-organizing cells while influencing cell behavior through physical and/or chemical stimuli, and the physical anchoring of cells to facilitate neovascularization. Additionally, pore size determines nutrient diffusion, oxygen concentration, and waste product expulsion, which are major determinants of shaping organoid morphology and function. Matrix stiffness can also influence stem cell maintenance and differentiation, while complex structures can guarantee the presence of ECM-bound structural proteins, such as laminin. The complex interaction between a defined scaffold architecture and the cultivation of organoid-forming stem cells can culminate in better cell maturation and organization as a result of mechanical stimuli [12, 13]. However, immature ECM can influence organoid formation, morphology, mechanical properties, and/or function, effects that should be decoupled. Approaches that disallow a structural cage, showing the significant impact of the ECM to organize cells, are allowed to develop, possessing only topical layers or proteins or functioning as barriers. It is also worth noting that, beyond their role in providing a 3D microenvironment, biomaterials

can also act as artificial inductive substrates to efficiently prompt direct differentiation towards a defined tissue type or shape. In addition to guiding spatial patterning, their rheology and chemistry can also be tuned to influence organoid function. As a result of technological advances in soft lithography and microfluidics, these 'bottom-up' approaches have ushered in new possibilities for creating complex 3D architectures with higher functional capabilities and mimicking key aspects of human tissues. However, several other features are also key determinants of the fate of organoids in mimicking disease: cell source choice and culture method [14, 15].

Biomaterials and Scaffold Design

The diversity of organoid morphology, composition, functionality, and developmental stage allows mimicking physiology and pathophysiology for a broad array of applications. Considering the wide range of compositions and functionalities present within the human body, diverse and innovative materials are required for the cost-effective fabrication of many unique organoid types. While some materials have wide general applicability, such as extracellular matrix-inspired hydrogels, the choice of scaffold material is still predominantly based on its ability to recapitulate the unique characteristics of the target tissue. Biomaterial selection depends on the required mechanical and biochemical properties, as well as biocompatibility, degradability, and mechanical stability during cell culture. Altogether, the choice of the biomaterial and the design of its derived scaffold critically influence organoid development. Below, we focus on these biomaterial requirements, and for each property, we place a strong emphasis on the characteristics that are important for the application of hydrogels in organoid engineering. The biochemical and mechanical properties discussed are determined either by the material itself or by the engagement of various chemical reactions and/or the exposure to environmental influences. This could be passive as well as more active and is crucial for in vitro cell behavior in the context of scaffold-based organoid culture. Finally, following a review of currently used materials suitable for organoid engineering, we evaluate the different kinds of smart materials that have the potential to reshape the field by better imitating cellular support. We also discuss their other potential advances and challenges, such as incompatibility with biological systems and issues relating to degradation rates [16, 17].

Cell Source and Differentiation Methods

The cell source, stem cells, and their source play a critical role in the successful engineering of organoids. There are three classical approaches to generating stem cells: (1) human adult stem cells, (2) embryonic stem cells, which originate from blastocysts, or (3) induced pluripotent cells, generating adult somatic cells for further reprogramming to a pluripotent state. By contrast, iPSCs present added advantages, such as a higher proliferative potential and fewer ethical concerns, but there is significantly less interest in studying the differences among iPSC-derived organoids. Different cells of origin would typically be used for the generation of different organoids. Nascent stem cells and their lineages play an important role in shaping organoid properties, followed by organoid position in the body. After starting to differentiate, about four cell types would be viable for somatic differentiation into organoids so that lineage apoptosis can be induced [18, 19]. Protocols for lineage commitment promote the appropriate specification and terminal differentiation of endoderm, ectoderm, and mesoderm precursor cells to produce the necessary cell types for organoid formation. Factors such as cell-cell signaling, growth factors, and cytokines control cell differentiation through the spatiotemporal tuning of signaling pathways required for tissue development. Specification and fine control of these pathways inform the in vitro differentiation strategies applied. To enhance differentiation, several external factors work synergistically with growth factors to ensure high efficiency of lineage specification. However, the development of reliable differentiation in vitro protocols is hampered by the variability between individual lines in differentiation protocols and organoid outcomes, emphasizing the requirement for fine-tuning and robust replication of these initial differentiation steps to ensure a reliable cell source for downstream research. Critically, the reliable functional integrity of resultant organoids is closely correlated with the quality of the cell source used in their generation, making it important to optimize and ensure the reproducibility of these early steps to improve the resultant organoid lines for use across several research fields. Although the cells are sources of organoid generation, improvements are still needed in several research areas, including the induction of hPSC differentiation, the activation of human ASCs, and the cellular lineage that is used as the root of the system protocol [20, 21].

Functional Characterization

The importance of including a functional characterization of an engineered organoid was fundamental in validating it as an adequate model. By functional, here we mean functions or characteristics inherent to

the mature tissue of interest, such as secretion of growth factors in cerebral organoids, electrophysiological activity in cortex organoids, or response to infection in liver organoids. Various techniques can be used to assess these functions in organoids, including gene expression analyses, electrophysiological characterizations, secretion assessments, or even metabolic profiling. Knowing that an organoid shows a similar metabolic profile to in vivo tissue does not validate the physiological relevance of the organoid, but is strong support, as organoids with differing genetic backgrounds will not show similarities, thus validating the characteristics of a particular organoid with a specific feature of interest. However, while meaningful, it remains most relevant to show that the organoid created faithfully replicates the tissue or organ of interest [22, 23]. The methodologies to prove this, for example, action potentials in nerve cells, bile secretion in liver cells, or hormone secretion in testicle organoids, are quite variable and generally not easily available in most standard laboratories. Furthermore, many times, standardized protocols to assess these characteristics have yet to be developed. This lack of standardized protocols might make it difficult to compare across studies whether a newly designed organoid performs functionally better or worse than previous studies. With this, understanding structural correlations, methods to validate functionality in organoids, the potential for such assays to guide further organoid design and common pitfalls, we urge researchers to consider functional characterization, if applicable, when embarking on new organoid or similar biological model engineering [24, 25].

Applications in Research

Most commonly, organoids are used to study the basic mechanisms of organogenesis and how these continue to operate in tissue renewal. For example, over the last decade, more than 30 studies have identified the various pathways involved in the development of the cerebral organoid. In constructing a tripartite structure mimicking the division of the nervous system, they have provided information on the differentiation, neurogenesis, and pattern formation involved in early brain development. Their diversity and accessibility have also made them key potential tools in the understanding of diseases and testing of new therapeutics. Disease modeling is perhaps the most wide-ranging approach that organoids have infiltrated, with frequencies in the study of such diseases as virus-induced microcephaly, obesity, and Alzheimer's disease. In the case of the latter, models have allowed for research into the protein aggregate disruption and amyloid/tau pathogenesis that is yet to produce relevant results in human clinical trials. Moreover, organoids can be made from multipotent stem cells derived from individual patients and would therefore serve as relevant test systems for genetics and potential drug treatments in the wider development of personalized medicine [26, 27]. This year, organoids have been grown from solid tumors, which exhibit a similar histological arrangement and mutations to the solid tumors in patients and respond reciprocally to chemotherapeutics in drug testing. Despite the research potential, however, organoids grow slowly compared to protoplasts or spheroids, with cysts requiring the longest time after plating. They also have variable outcomes that are dependent upon the conditions imparted during the induction stage, with a molecular disparity resulting in the growth of either structures that mimic early embryonic structures or those that follow to generate more complex organoids. Similar to adult stem cells, expanding retinal organoids was similarly difficult with the appropriate mitogens, but conducive to the initiation of a neurosphere and the resulting differentiation of rods and other retinal cells. Organoids also have a limited size range, with the surface area of the apical side limiting the distance oxygen can diffuse from the medium to the center of the structure. They also develop significant necrotic cores as they begin to unfold. There are also concerns about their proposed mats for high-throughput drug testing, as the readout methods often disrupt the delicate culture maintaining the organoid's form or gene expression. They also suffer from a degree of donor variability in both size and form [28, 29].

CONCLUSION

The engineering of functional organoids represents a frontier in biomedical research with transformative potential for understanding human development, modeling diseases, and advancing drug discovery. Despite remarkable progress, challenges such as limited scalability, functional variability, and incomplete replication of in vivo tissue environments remain. Innovations in biomaterials, scaffold design, and differentiation protocols are essential to overcoming these limitations. Furthermore, the standardization of functional characterization techniques will enable reliable comparisons and reproducibility across studies. As the field progresses, interdisciplinary collaboration among biologists, engineers, and material scientists will be critical to developing robust, scalable, and physiologically relevant organoids. Such advancements could revolutionize personalized medicine, regenerative therapy, and drug screening, bringing the promise of organoid technology closer to clinical and research applications.

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