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Unleashing the power of biomaterials to enhance organoid differentiation and function

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Abstract

Biomaterials are revolutionizing organoid development by offering tunable platforms that provide instructive cues, which enhance cell fate transitions, tissue-level functions and reproducibility. These advances are crucial for harnessing the translational potential of organoids.

Organoids are miniature, 3D in vitro models often, developed from stem cells, that recapitulate aspects of an organ's structure and function in vivo. Advances in organoid development and their applications in disease modeling are poised to improve our understanding of physiological and pathological processes and transform the drug development pipeline, bringing life-saving therapeutics to patients more efficiently.

Current approaches to generate organoids often result in dense, heterogenous cell clusters that display immature or fetal-like tissue characteristics with restricted and irreproducible patterning, vascularization and function. Organoid differentiation and self-organization from stem cells are stochastic processes, leading to variability across replicate experiments, which is compounded by the use of poorly defined cell culture and differentiation reagents such as fetal bovine serum and Matrigel. Additionally, tissue–tissue interfaces in organoid models often lack sufficient structural support to promote in vivo-like cell–cell and cell–matrix interactions, leading to challenges in accurately modeling interfaces such as the glomerular basement membrane in the kidney, alveolar–capillary membrane, blood–brain barrier and blood-retina barrier. Recent organoid research has begun to address these issues through the innovative use of biomaterials (Fig. 1).

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Author contributions

S.M. conceived the idea and strategy for this work. S.M and H.A. wrote the manuscript. H.A. developed the original sketch for the illustration in Fig. 1. S.M. supervised H.A.

Competing interests

The authors declare no competing interests.

Biomaterials can exhibit inert or bioactive characteristics, but their use in biomedical applications was originally driven by their inert properties, as the first biomaterials served as structural supports in applications such as sutures, prosthetics, dental fillings and contact lenses. We previously showed that cells can alter their gene regulation and behavior (for example, cell growth, differentiation, patterning or pluripotency) in response to mechanical and biochemical cues from biomaterial surfaces[1]. We believe the use of these biomaterial capabilities to guide the formation of functional organ-specific tissues within multicellular organoids remains underexplored.

In vivo, cells interact with the surrounding extracellular matrix (ECM), which provides structural support as well as biochemical and mechanical signals that are important for cell function and whose disruption has been implicated in disease progression. To facilitate organoid development using biomaterials, stem cells can be cultivated in engineered 3D matrices such as porous hydrogel scaffolds and electrospun protein-based fibers to create extracellular environments that mimic native ECM. An exemplary instance is the modular hydrogel system developed by Gjorevski et al., which creates an environment initially conducive to the proliferation of intestinal stem cells[2]. As time progresses, the hydrogel undergoes gradual transformations, enhancing conditions for differentiation and subsequent development of intricate intestinal organoids. The successful development of such a defined biomaterial system as an alternative to animal-derived matrices paved the way for the robust culture of human stem-cell-derived organoids.

The development and use of organoids in research has gained significant momentum since the 2009 report by Sato et al.[3] Stem cells, including pluripotent stem cells, are now widely used to generate organoids because of their remarkable capacity for self renewal and differentiation into desired cell types when provided appropriate biochemical and biophysical signals. In our experience and upon critical review of existing literature, organoid differentiation and self-organization from stem cells exhibit high stochasticity, leading to variable structures and patterns across replicate experiments. Further limitations include batch-to-batch differences in organoid shape, size, cellular composition and function. These variations contribute to the reproducibility challenges within the field. Factors that contribute to these issues include the pervasive use of poorly defined cell culture and stem cell differentiation reagents such as fetal bovine serum, knockout serum replacement and Matrigel (an ECM with undefined composition derived from mouse tumors)[4]. The batch-to-batch disparities in these reagents' molecular and structural makeup pose challenges for achieving precise control over organoid differentiation quality and replicability. We believe that in the case of Matrigel, the molecular composition and the presentation of biochemical, biophysical and spatiotemporal cues to cells are nearly impossible to replicate between technical or independent experiments, which contributes to the substantial production of off-target cells, adversely impacting organoid development, patterning and organ-specific physiological relevance. Furthermore, most organoid culture protocols employ undefined soluble serum components and other recombinant growth factors or small molecules (with off-target effects) in a manner that lacks spatiotemporal control. Considering the critical role of spatiotemporal ligand distribution within the ECM in controlling cell signaling — whether in an activating, inhibitory or chemotactic capacity — it is essential to precisely define the molecular signals necessary for achieving specific cell

fate outcomes and to present these signals to cells in effective formats to advance organoid research.

Biomaterials produced from natural polymers (for example, hyaluronic acid and alginate) or from synthetic polymers (for example, polyacrylamide and polyethylene glycol) are being used to transform organoid differentiation strategies by tuning their mechanical and biochemical properties to elicit specific cellular responses. Polymeric biomaterials have been tuned to exhibit variable stiffness, topography, viscoelasticity, ligand density, biodegradability and surface chemistry[1,5]. Such tailored properties can stimulate specific cell-signaling pathways and guide stem cell differentiation toward desired tissue-or organ-specific cell types, facilitating the generation of functional organoids. For example, Pérez-González et al. cultured mouse intestinal organoids on hydrogels with different stiffnesses and showed that biomaterial stiffness influences epithelial compartmentalization, crypt folding and collective cell migration in the intestinal epithelium[6]. In an example of biomaterials steering organoid patterning, Nerger et al. demonstrated that hydrogel viscoelasticity influences the formation and composition of nephron segments in stem cell-derived kidney organoids[7]. Intriguingly, the authors also observed that accumulation of extracellular calcium in the culture medium, which can be influenced by the hydrogel scaffold, reduced the ratio of glomerulus-to-tubule compartments in the developing kidney organoids. Together, these examples underscore the role biomaterials can play in guiding cell signaling, stem cell differentiation, tissue patterning and organ-level responses.

Future perspective

Advances in biomaterial design and engineering are enhancing the development of organoids because biomaterial features can be finely tuned to synergistically guide tissue development and specialization. For example, polymeric hydrogels or electrospun protein fibers can be functionalized with ECM proteins or peptides to mimic in vivo-like molecular loading, release mechanisms and spatiotemporal display to activate desired biochemical signaling pathways[8]. Cross-linking technologies including stimuli-responsive crosslinkers that respond to light, specific enzymes, solution pH, temperature, redox reactions and other small molecules can be used to synthesize biomaterials with multimodal signaling capabilities or trigger cascading reactions that are programmed by user-defined Boolean logic control systems[9]. We anticipate that successful implementation of these innovative and iterative design approaches will offer unprecedented opportunities to create customizable biomaterial systems for robust patterning and specialization of organoids in the future.

Additionally, biomaterials have the potential to address the limited cell organization in organoids, which contributes to their failure to closely mimic complex in vivo tissue structures. For example, kidney organoids consist of vascular endothelial and glomerular epithelial-like cells, but their functional interface at blood filtration capillaries is non-existent in current organoid models. Similarly, the alveolar–capillary membrane, blood–brain barrier and blood–retina barrier are all challenging to develop using traditional organoid differentiation methods. To overcome some of these challenges, biomaterials such as porous polymeric membranes, electrospun fibers and 3D hydrogel systems have been used to engineer the basement membrane components found in native tissues[10,11]. We

expect coupling these bioengineering strategies with co-culture systems to provide effective strategies for promoting in vivo-like cell–cell and cell–ECM interactions and paracrine signaling, which are crucial determinants of tissue formation and organ physiology.

Improving organoid models also requires addressing current deficiencies in recapitulating well-connected vascular networks and other fluidic circuits that are essential for biological processes such as detoxification, nutrient transport and cell signaling. Because organoids are usually hundreds of micrometers thick, they develop necrotic regions, particularly in the core, owing to limited innervated vasculature and oxygen and nutrient supply. We speculate that the limited nutrient diffusion and waste removal in organoids can be addressed by using hydrophilic biomaterials (for example, hydrogels) with high diffusivity. Layer-by-layer 3D printing and cell-laden fiber techniques can be leveraged to generate biocompatible cellular units that can be integrated to create 3D tissue constructs with precise cellular organization and compartmentalization, along with immobilized signaling epitopes. Strategic incorporation of endothelial cell-laden fibers within parenchymal cell-containing fibers can be used to facilitate the generation of functional microvascular networks within organoid constructs[12]. We and others have demonstrated the use of microfluidic devices[13,14] to control the flow of nutrients, oxygen and waste products, mimicking natural blood flow in organs; this can be applied to enhance organoid growth, viability and function, as demonstrated in the use of microfluidics in kidney organoid tubules [15]. Leveraging these biomaterial-based approaches will transform organoid differentiation methods and lead to pivotal milestones in engineering more accurate organoid models for biomedical and translational applications.

As organoid research advances toward applications in translational and precision medicine, it is paramount for researchers to continue to enhance organoid development, patterning and function by integrating organoids with cutting-edge technologies and advances in biomaterials, microfluidic systems, 3D bioprinting, proteomics, CRISPR–Cas9 and other genome editing technologies, molecular bar-coding technologies, and computer modeling. Organoids emerge from unspecialized stem cells to increasingly specialized cells and patterned tissues, and future biomaterials could be designed to provide dynamic molecular and biophysical cues to promote real-time modulation of organoid phenotype and function — for example, by using molecular logic gates with user-defined Boolean controls for on-demand perturbations to improve organoid development.

Although the application of artificial intelligence (AI) in predicting and guiding biological processes in vitro and in vivo is still in its early stages, we anticipate that interdisciplinary strategies that leverage AI, modular biomaterial systems and microphysiological systems hold the potential to transform the field of organoid research and human disease modeling.

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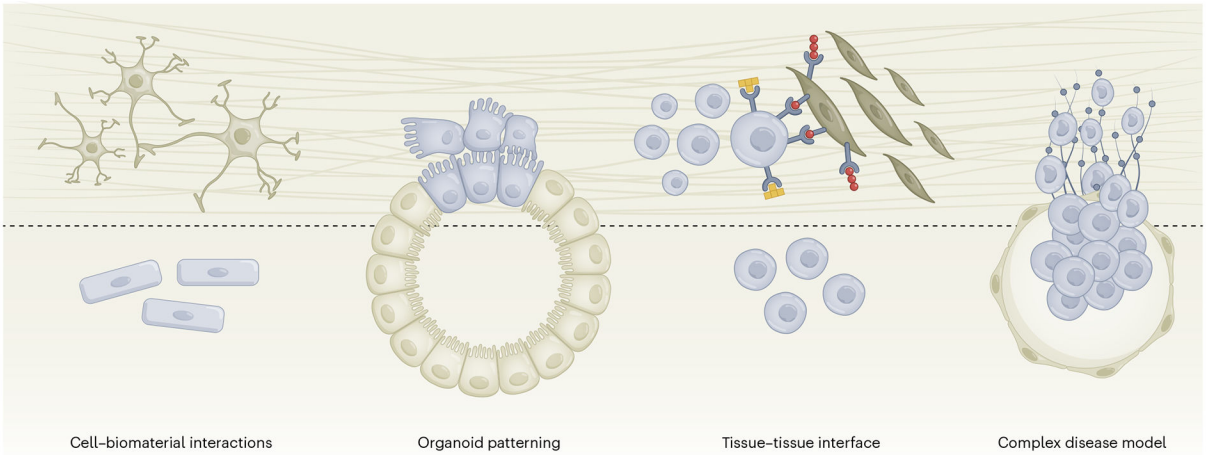

Fig. 1 |. Biomaterials influencing organoid development and tissue structure.
Engineered biomaterials play an essential role in organoid development by directing stem cell fate and morphogenesis to establish in vitro organoids with in vivo-like cellular composition and architecture. The bottom panel depicts the cells or organoid structures without instructive biomaterials. In contrast, the top panel illustrates how cells and organoid structures can be influenced by biomaterials (depicted as fiber-like structures). Bottom (from left to right): undifferentiated cells, an unpatterned organoid structure, cells without complex cell–cell interactions, and non-invasive tumor cells within the tissue. Top (from left to right): fully differentiated cells (using neurons as an example), organoid structure with a specific pattern, cell–cell interactions between two different cell types, and invasive tumor cells exhibiting complex matrix production and signaling molecule activity.