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Review article

Cellular self-assembly and biomaterials-based organoid models of development and diseases

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ABSTRACT

Organogenesis and morphogenesis have informed our understanding of physiology, pathophysiology, and avenues to create new curative and regenerative therapies. Thus far, this understanding has been hindered by the lack of a physiologically relevant yet accessible model that affords biological control. Recently, three-dimensional *ex vivo* cellular cultures created through cellular self-assembly under natural extracellular matrix cues or through biomaterial-based directed assembly have been shown to physically resemble and recapture some functionality of target organs. These "organoids" have garnered momentum for their applications in modeling human development and disease, drug screening, and future therapy design or even organ replacement. This review first discusses the self-organizing organoids as materials with emergent properties and their advantages and limitations. We subsequently describe biomaterials-based strategies used to afford more control of the organoid's microenvironment and ensuing cellular composition and organization. In this review, we also offer our perspective on how multifunctional biomaterials with precise spatial and temporal control could ultimately bridge the gap between in vitro organoid platforms and their *in vivo* counterparts.

Statement of Significance

Several notable reviews have highlighted PSC-derived organoids and 3D aggregates, including embryoid bodies, from a development and cellular assembly perspective. The focus of this review is to highlight the materials-based approaches that cells, including PSCs and others, adopt for self-assembly and the controlled development of complex tissues, such as that of the brain, gut, and immune system.

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1. Introduction

Organogenesis and morphogenesis involve the formation of complex tissue and organs from single cells. These intricate processes include orchestrated yet complex and dynamic chemical and physical signals that influence the self-assembly of non-prepatterned cells into the target tissue or organ. Biologists and bioengineers have studied both processes to better understand the mechanisms of development, organ physiology, and pathophysiology to inform curative and regenerative therapies [1–3]. In the past, models of organogenesis have included regular and genetically engineered *in vivo* systems, but these models suffer from limited accessibility, complexity, and often restricted multi-dimensional and multiscale biological control. Also, several conditional knockouts are embryonically lethal, further imposing limitations on understanding cell development [4,5]. On the other hand, conventional two-dimensional (2D) in vitro models are often an oversimplification and are unrepresentative of the three-dimensional (3D), multi-cellular natural milieu of typical target organs. Recently, organoids have shown potential for bridging the high-throughput, cost-effective nature of in vitro systems with the physiological relevance of *in vivo* models, as indicated in Fig. 1A and described in Table 1 [6–16].

Organoids, as defined here, are *ex vivo* three-dimensional (3D) cellular structures that either self-organize or are directed to assemble under specific organogenesis cues; these structures must also physically resemble, either fully or partially, the architecture, cellular organization, and composition of an *in vivo* organ while recapturing partial or complete functions of the organ. Organoids can be engineered based on secreted soluble signals, non-modular extracellular matrix signals, and modular extracellular matrix. A comparison of these methods across throughputness, tenability, stochasticity, patterning, and scalability is presented in Fig. 1B. Thus far, a major focus of organoids research has been on pluripotent stem cells (PSCs), which, when grown in a 3D microenvironment, self-organize and acquire physiologically relevant cellular patterning to develop into several endoderm- and ectoderm-derived tissues, often mimicking their *in vivo* counterparts. Several elegant reviews have highlighted PSC-derived organoids and 3D aggregates, or embryoid bodies, from a development and cellular assembly perspective [12,14,17,18]. The focus of this review is to highlight the materials-based approaches that mammalian cells, including PSCs and others, adopt for self-assembly and controlled development of complex tissues, such as that of the brain, gut, and immune system. In this review, we first discuss the organoids that result from self-assembly of cells under secreted and/or exogenous growth factors or natural extracellular matrix (ECM) instructed cues (e.g. Matrigel), and review the emergence of these organoids as materials with novel and important properties. We next emphasize the role of semi-synthetic and synthetic biomaterials in controlling assembly of organoids. We extensively discuss both approaches for three organoid models – intestine, brain, and immune – in the context of modeling development and disease. These models are emphasized because of their complexity and upcoming efforts in biomaterials organoids. We finally provide perspective on how new biomaterials with spatial and temporal control of bio-ligand signaling could guide tissue dynamics, morphogenesis, vascularization, and organogenesis, *ex vivo*.

2. A materials approach to self-assembly of cells

The flow of information between cells and their neighboring microenvironment is often considered bidirectional, leading to a diverse set of biological phenomenon, including the assembly of tissue structures and organs. The pioneering work of Mina Bissell and colleagues has established an understanding of how the mechanical and chemical composition of the extracellular matrix regulates cell behavior and function in malignant cells [19–22]. Likewise, organoid models have used animal derived-ECM networks, such as Matrigel [23], collagen [24], and other basement membrane matrices (e.g. GelTrex [25]), to influence cellular self-assembly and tissue formation. Within Matrigel platforms, myriad organoids have been developed, and key functional and pathological mechanisms have been elucidated [26–28]. Although Matrigel ultimately enhances the self-assembling capacity of PSCs, likely because of the complex distribution of nutrients and protein gradients, this strength can also serve as the platform's major weakness due to the ensuing complexity [23,29], compositional variability [30–32], and lack of control over individual parameters, such as ECM ligands (Fig. 1). Within these platforms, cells often assemble into heterogeneous organoids in terms of viability, size and shape [33], and suffer from relatively random spatial positioning of tissue regions. Importantly, the cocktail of growth factors and signaling cascades in Matrigel work simultaneously, which may inhibit complementary pathways, making it difficult to tune and control the signal transduction in cells undergoing organogenesis. Finally, animal-derived hydrogel materials present a major roadblock in clinical application of organoids because of the possibility of pathogen transfer and immunogenicity.

An ability to sculpt the biophysical and biochemical microenvironment with control over signaling milieu may help increase the faithfulness of stem cell- and progenitor-derived organoids to real organs, and open up the possibility to guide morphogenesis and organogenesis in culture. To meet this need, the uses of biomaterial-based synthetic or recombinant ECM analogs have recently gained momentum to provide a more precise and better control of the organoid's microenvironment [34–37]. As summarized in Table 1, organoids have already captured several key organ functions and elucidated differences in developmental and dysfunctional mechanisms as well as in interactions with therapies when compared to conventional 2D cell culture models. Of these models, organoids using both 3D cellular self-assembly and biomaterial-controlled microenvironment approaches have been established for the intestine, secondary lymphoid organ, and brain, and therefore, we discuss them in greater detail than other organoid models. A summary of different types of biomaterials used to engineer these three organ systems and their effect on organoids is provided in Table 2. In the following sections, we describe the components and function of each these three organs, followed by the cellular self-assembly and biomaterial based organoid methods used to study development and dysfunction. This development of ex vivo organoid platforms opens up many exciting translational opportunities in basic science, drug discovery, deep sequencing efforts for personalized medicine, and will likely be critical for the emerging cell and tissue biomanufacturing sector (Fig. 1C). The organoids can facilitate de