3D Bioprinting of Human Brain Organoids

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Brain organoids are invaluable tools for pathophysiological studies or drug screening, but there are still challenges to overcome in making them more reproducible and relevant. Recent advances in three-dimensional (3D) bioprinting of human neural organoids is an emerging approach that may overcome the limitations of self-organized organoids. It requires the development of optimal hydrogels, and a wealth of research has improved the knowledge about biomaterials both in terms of their intrinsic properties and their relevance on 3D culture of brain cells and tissue.

Keywords: biomaterials ; brain organoid ; 3D bioprinting ; scaffold

1. Introduction

Neurological disorders are the leading cause of disability and the second cause of deaths worldwide, and this burden is expected to be driven up by population aging [1]. Despite considerable progress in medical imaging, the complexity and inaccessibility of the brain still hinder research on the live organ. Post-mortem explorations of brain samples have provided significant insight, but their potential to pathophysiology or drug screening studies is obviously limited. Even though they have provided so much knowledge about brain biology, mouse models do not always properly recapitulate human neurological disorders because of the significant differences in development, structure and physiology of rodent and human brains [2][3]. Classical monolayer (two-dimensional, 2D) cultures of neural cells have unveiled important knowledge on brain disorders with genetic or infectious aetiology (as in the case of lissencephaly [4][5]), but they cannot recapitulate the complex events underlying brain development or homeostasis. Hence, there is an urgent need for new in vitro models. Three-dimensional (3D) cell culture has proven its multiple benefits compared to 2D culture in terms of cell function and homeostasis, and has paved the way for human brain organoids [6]. Since the pioneering work of Lancaster et al. [7], and throughout the last decade, the generation of brain organoids from human induced pluripotent stem cells (iPSCs) or human embryonic stem (ES) cells was a milestone towards modelling healthy or diseased human brain and provided a wealth of knowledge on brain pathophysiology [8]. However, there are still challenges to overcome in making organoids more reproducible and relevant to the complexity of the brain; in particular, brain organoids fail to reproduce cerebral substructures and lack microvasculature [9]. Meanwhile, tremendous advances in 3D bioprinting of live tissues or organs have opened up new horizons for disease modelling in recent years. Three-dimensional bioprinting consists in the precise and automated deposition of cell-laden hydrogels, so-called “bioinks”, for the biomanufacturing of complex human living tissues or organs, including neural tissues and, expectedly, brain. Convergence between organoid technology and 3D bioprinting is expected to open new avenues in brain research. The development of optimal hydrogel biomaterials for the bioprinting of neural organoids is of utmost importance and has been the subject of an increasing amount of work in recent years. A whole field of research has improved the knowledge about biomaterials both in terms of their intrinsic properties and the impact of their use on 3D brain cells and tissue culture. A wealth of recent and very informative articles have cleverly reviewed brain mechanobiology [10] or mechanical properties of biopolymers [11][12][13]. Meanwhile, there is a lack of review articles that focus on the biological roles that these biomaterials exert on the enladen cells. Biomaterials are rarely biologically neutral. they are able to deliver or collect biological signals to or from the cells, provide cells with adhesion sites and shape cellular microenvironments. Hydrogel biomaterials direct cell differentiation depending on their matrix stiffness and may potentially induce organogenesis through mechanotransduction [14][15]. Furthermore, the mechanical properties of the cell microenvironment are involved in normal brain tissue function but also in neuropathological situations [10]. Although of crucial importance, matrix elasticity has been poorly investigated in 3D culture of brain organoids, as these were mainly produced using Matrigel™, a basement membrane matrix secreted by mouse sarcoma cells. It is hypothesized that defined hydrogel biomaterials may provide a more reproducible cellular microenvironment to direct stem cell proliferation and differentiation than animal-derived and variable extracellular matrices to produce brain organoids. In combination with 3D bioprinting, the multiscale complexity of brain structures may be mimicked.

2. Brain Organoids: Potential and Limits

Brain organoids are 3D clusters of cell populations derived from primary tissue, embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs), capable of self-renewal and self-organization, and that recapitulate certain organ functionality [16]. Brain organoids harbour brain-like substructures, allow for neuronogenic or gliogenic differentiation, and exhibit electrophysiological activity indicative of neuronal network level functioning [17]. Organoids generated from induced pluripotent stem cells can model complex neurological disorders such as those from patients with Rett syndrome, which show abnormal, epileptiform-like activity [18]. This makes organoids invaluable tools for pathophysiological studies or drug screening. Since the first human brain organoid described by Lancaster [7], several studies have pushed the limits of what was thought possible. Neurons represent a non-homogeneous network of cell populations with molecular, regional, and functional specificities showing different sensitivities to disease. Specific procedures, so-called “guided” procedures, have been reported to drive organoid patterning towards distinctive lineages [19]. For instance, organoids with high density in hippocampal [20], cortical [21], dopaminergic [22], glutaminergic [23], gamma-aminobutyric acid (GABA)-ergic [23] neurons, as well as retinal ganglionic neurons [24], astrocytes [25], microglia [26][27] or oligodendrocytes [28], have been produced, to mention a few ground-breaking examples. Furthermore, organoids which recapitulated bilateral optic vesicles [29] and vasculature-like structures [30][31] have been reported.

Against all odds, the fascinating ability of organoids to organize themselves spontaneously raises true issues [9]. Firstly, scalability issues hamper their use in high-throughput assays such as drug screening, since the volume of organoids can exceed one cubic millimetre upon maturation [32]. Moreover, organoid spatial organisation is largely unpredictable and thus hampers the reproducibility of the 3D models. Furthermore, while the diversity and distribution of cell types in organoids have strong similarities to those of embryonic or foetal tissue, the spatial organisation of cellular components and paracrine signals remain far from nature. In particular, axial patterning of soluble morphogens requires topological patterns that simply do not exist in organoids. Organoids cultured in suspension exert no mechanical constraint driving neural tissue development, as is the case in vivo (for example as imposed by the developing skull) [33]. Inconsistency might result in heterogeneity and phenotypical variability of organoids, possibly overlapping or even hiding caused by the disease modelled [34]. Controlled assembly of pre-differentiated organoids [35] is a clever approach to bypass this problem, but it offers limited room to achieve standardization of a model amenable to reproducible assays or controlled geometries. Furthermore, the innermost cell populations within organoids hardly have access to oxygen and nutrients present in the culture medium, which inevitably results in local necrosis with possible release of soluble mediators impacting the rest of the organoid. This is strongly contributed by the absence of vascularizing structures, which negatively impacts progenitor populations [36] and further contributes to experimental variability [34]. Current strategies to overcome this pitfall rely on culture in bioreactors, transplantation into mouse models or perfusion with microfluidic devices [37].

3. Three-Dimensional Bioprinting: Benefits and Challenges

Three-dimensional (3D) bioprinting is a highly promising technology for both tissue regeneration and organ replacement but may certainly help in the short term to develop models for studying human organ development and diseases as well as for drug screening in vitro as alternatives to animal experiments [38]. Briefly, bioprinting consists in the computerized fabrication of 3D structures composed of living cell-laden biocompatible hydrogels layer by layer. Three-dimensional bioprinting allows different cell types to be distributed and arranged in any possible pattern, offers high reproducibility since it is computer-driven, and favours accessibility of cells to nutrients and oxygen because it controls the pattern infill in the 3D tissue engineered constructs. It also allows for pre-differentiation of cell populations before controlled assembly by using bioprinting, a so-called “bottom-up” approach. For instance, 3D bioprinting allows for the accurate picking and assembly of whole cell spheroids into higher-order structures with minimal cell damage [39]. Furthermore, 3D bioprinting offers a simple solution to reproduce gradients of morphogenic molecules such as those involved in development, by bioprinting cell populations in distinct but contiguous hydrogels containing the appropriate soluble factors at different concentrations. Finally, 3D bioprinting makes it possible to insert structures recapitulating blood vessels or even vascular networks inside an organoid, by using sacrificial bio-inks, which are intended to dissolve to leave room for the “vessel” lumen [40].

Thus far, 3D bioprinting of brain organoids has been limited by technological challenges such as printability of hydrogel bioinks, shape fidelity of 3D constructs post fabrication and limited cell migration and/or differentiation [41][42][43]. Its development is the subject of much research at the technological level but also in terms of the hydrogels used as bioinks. These biomaterials should not only be cytocompatible to support cell adhesion, growth and differentiation, but they must have adequate viscoelastic properties for a continuous micro-extrusion as well as forming cross-links to ensure the stability of the 3D constructs in culture [44]. Most hydrogels having favourable biological properties to support brain organoids culture do not meet the physicochemical requirements for 3D bioprinting (e.g., native collagen, fibrinogen,

Matrigel). For instance, extrusion bioprinting requires shear shinning and high viscosity properties to ensure a continuous and stable strand extrusion of the bioink at physiological temperature, pH and osmolarity. For these reasons, blends of hydrogels are often used to improve shear thinning properties of bioinks. They result, however, in lower ability to support neurogenesis and brain organoid maturation. Furthermore, components of the brain extracellular matrix are often chemically modified by methacrylate coupling (e.g., gelatine Methacrylate, GelMA) to form cross-links by photopolymerization and to render the 3D constructs stable in culture. Nevertheless, photocurable bioinks induce some cell mortality due to the presence of free radicals. A large number of solutions have been tested in recent years to improve bioink properties in terms of printability, long-term stability, cell compatibility and organoid maturation, using raw or defined, homogeneous or heterogeneous biomaterials, should they be chemically modified or not. Recent works have revealed that unmodified polymers can efficiently support bioprinting of neural tissues, and researchers here focus on such native molecules. Most of them are components of the brain extracellular matrix, a key structure to understand the outcomes of the microenvironment on cell and tissue homeostasis.

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