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Design of neural organoids engineered by mechanical forces

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ABSTRACT

Neural organoids consist of three-dimensional tissue derived from pluripotent stem cells that could recapitulate key features of the human brain. During the past decade, organoid technology has evolved in the field of human brain science by increasing the quality and applicability of its products. Among them, a novel approach involving the design of neural organoids engineered by mechanical forces has emerged. This review describes previous approaches for the generation of neural organoids, the engineering of neural organoids by mechanical forces, and future challenges for the application of mechanical forces in the design of neural organoids.

1. Introduction

Neural organoids present three-dimensional (3D) miniature brain-like structures that are derived from pluripotent stem cells (PSCs), such as embryonic stem cells or induced pluripotent stem cells (iPSCs) (Kadoshima et al., 2013; Lancaster et al., 2013). Neural organoids recapitulate the features of the human brain at the early stage, therefore offering several advantages for studying early human brain development and related diseases. First, this technology allows researchers to create three-dimensional architecture that carry the genetic background of individuals with specific neurological disorders, providing a valuable tool for studying disease-specific mechanisms and provide opportunities for disease-pathomechanism analysis and drug discovery. Additionally, neural organoids can be genetically modified and/or exposed to external factors such as viral infections, noxious substances, and drug treatments, providing insights into how these factors affect brain morphogenesis and function (Di Lullo and Kriegstein, 2017; Amin and Pasca, 2018).

While neural organoid engineering is progressing rapidly and is leading to a new era of brain science research, there is still a long road ahead for the development of the highly complex organoids such as the human brain. In this review, we summarize up-to-date information about achievements in the field of neural organoid technology and discuss the potential of the next-generation neural organoids with the application of mechanical forces for organoid design.

2. Previous approaches to neural organoid generation

Neural organoids technology typically involves a series of steps to recapitulate the sequential events that occur during embryogenesis and human brain development. In general, the creation of organoids, and also neural organoids, is based on a combination of spatial, biological, and synthetic design strategies that closely resemble natural organs in structure and function (Takebe and Wells, 2019). Size control and composition of cell aggregates as well as modeled tissue-tissue interaction create an initial physical arrangement and spatial organization that allows for self-organization and the emergence of complex tissue structures. Biological environmental control refers to the deliberate selection and implementation of environmental factors that mimic the complex conditions found in vivo during organogenesis, homeostasis, and regeneration. This approach involves carefully designing the culture conditions and providing specific cues such as inducing factors or extracellular matrix (ECM) components to create an environment that could recapitulate the biological complexity of living organisms.

To date, whole neural organoids that include various brain regions (Lancaster et al., 2013) or regionally isolated neural organoids have been generated from human PSCs including cerebral (Kadoshima et al., 2013; Qian et al., 2016), cerebellum (Muguruma et al., 2015), midbrain (Jo et al., 2016; Qian et al., 2016; Monzel et al., 2017), hippocampus (Sakaguchi et al., 2015), thalamus (Xiang et al., 2019), hypothalamus (Qian et al., 2016), choroid plexus (Pellegrini et al., 2020), spinal cord (Ogura et al., 2018; Lee et al., 2022), and neuromuscular organoid

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(Faustino Martins et al., 2020). Even a complex process like somite segmentation has been recapitulated in 3D structure (Miao et al., 2023; Yamanaka et al., 2023). Besides, cultured neural organoids also exhibit maturation phenotypes such as gliogenesis and myelination (Pasca et al., 2015; Sloan et al., 2017; Madhavan et al., 2018; James et al., 2021).

Based on the achievements from the first generation of neural organoids, many different approaches have been taken to overcome the existing limitations of the organoid system such as reproducibility and variability, absence of blood vessels as well as immune system, and low-maturation (Hofer and Lutolf, 2021). These complicated approaches involve integrated glial cells such as oligodendrocytes (Madhavan et al., 2018; Marton et al., 2019), microglia (Abud et al., 2017; Ormel et al., 2018; Song et al., 2019), and blood vessels (Cakir et al., 2019; Ham et al., 2020; Shi et al., 2020) into neural organoids. Culture sectioned neural organoids at the air-liquid interface also showed enhancing maturation and functioning (Giandomenico et al., 2019; Qian et al., 2020). Another popular engineering approach is the combining of different region-specific neural organoids into fusion or assemblies to recapitulate the interaction between brain regions. The fused organoids can mimic neural migration, projection, or functional neural circuits (Bagley et al., 2017; Birey et al., 2017; Xiang et al., 2017; Sloan et al., 2018; Xiang et al., 2019; Andersen et al., 2020; Miura et al., 2020; Ao et al., 2021; Miura et al., 2022). Remarkably, connected neural organoids through projecting axons (also called connectoids) in a specific microdevice (Kirihara et al., 2019; Osaki and Ikeuchi, 2021) or within a tube of gel (Cullen et al., 2019) can generate more complex neural circuits. On the other hand, a complex technique such as creating a concentration gradient of specific morphogens within neural organoids has been successfully simulated to mimic patterned structures during brain development (Cederquist et al., 2019; Rifes et al., 2020; Seo et al., 2023).

3. Engineering neural organoids by mechanical forces

During brain development, mechanical forces play important roles that impact brain morphology and function (Javier-Torrent et al., 2021). For example, cerebrospinal fluid (CSF) flow during embryogenesis shows critical roles in several important processes such as radial glial cell polarization, neuronal migration, axon growth and guidance as well as cortical folding events (Sawamoto et al., 2006; Guirao et al., 2010; Ohata and Alvarez-Buylla, 2016; Garcia et al., 2018). Moreover, the flow of CSF is a key signal for neural stem cell renewal through the activity of the epithelium sodium channel (Petrik et al., 2018). Besides, substrate properties such as stiffness and topography also regulate neural growth, migration, and outgrowth (Tanaka et al., 2018; Leclech et al., 2019). Emerging evidence has shown that impairment of brain mechanical properties such as brain stiffness is associated with neuronal disorder (Hiscox et al., 2020; McIlvain et al., 2020). In addition, the role of mechanical forces in controlling cortical folding has been noticed (Kroenke and Bayly, 2018). While the mechanisms of cortex folding are large unknown, the contribution of some ECM components is required for this process (Long et al., 2018). Recognizing the importance of mechanical forces in embryogenesis and organogenesis, involving these factors in neural organoid technology, is a promising strategy for improving the reliability of the neural organoid system.

3.1. Mechanosensing and mechanotransduction in brain physiology

During organogenesis, a variety of intrinsic forces as well as extrinsic forces were found to guide early embryo development. Forces can arise from various sources, including cell-generated forces, mechanical interaction between cells and the surrounding extracellular matrix, tissue growth, as well as externally applied forces such as shear, tension, or compression (Vining and Mooney, 2017). The processes that cells recognize and adapt to environmental forces and convert into biochemical signals are called mechanosensing and mechanotransduction. These activities involve a complex network of proteins, receptors, ion channels, and signaling pathways.

At the cellular level, intrinsic forces, such as actomyosin motor during cell division, are sensed and transferred to other cells via transmembrane receptors as integrins and cadherins that connect the ECM to the cell cytoskeleton (Kechagia et al., 2019; Angulo-Urarte et al., 2020). ECM cues such as stiffness and tension can modulate these proteins' structure, followed by influencing the cytoskeletal organization and activate downstream signaling pathways that control several cellular functions such as focal adhesion kinase (FAK) (Chaturvedi et al., 2007) and YAP/TAZ signaling (Voltes et al., 2019). For example, stiff or soft matrices facilitate the neuronal/glial fate in the regulation of Rho GTPase signaling (Sampayo et al., 2023). Evidence showed that the mechanical forces generated during zebrafish hindbrain segmentation have an impact on controlling the proliferation and differentiation of neuronal progenitors through the activity of YAP/TAZ proteins (Voltes et al., 2019).

On the other hand, extrinsic forces from the environment such as shear stress are recognized from multiple pathways including mechanosensitive ion channels, flow-sensitive proteins, and primary cilium that are located on the cell surface. Several mechanically activated ion channels were discovered as sodium ion channels (DEG, ENaC, ASIC), transient receptor potential ion channels (TRPN, TRPV4, TRPA1), potassium channel subfamily K members (TREK1/2, TRAAK), and Piezo channel family (Piezo1/2) (Ranade et al., 2015). The open or closed status of these channels allow the flow of ions across the membrane, leading to changes in membrane potentials and intracellular calcium levels. Human neural stem cells exhibit stretch-activated ion channels Piezo1 and their activity influences neuronal/astrocytic specifications by adapting to substrate stiffness (Pathak et al., 2014). Moreover, neural stem cells at brain ventricles sense the flow of CSF through their epithelium sodium channels (ENaC), thereby regulating their proliferation (Petrik et al., 2018).

In addition, forces can be sensed by primary cilia, the sensory organelles extending from the surface of most mammalian cells. Primary cilia are found in various cell types in the human brain and play important roles in the nervous system (Guemez-Gamboa et al., 2014). Primary cilia actively respond to mechanical cues such as fluid flow, matrix stiffness and biological forces by adjusting their length (Besschetnova et al., 2010), their frequency and their position (Williantarra et al., 2022). There are various receptors, ion channels, and signaling molecules that localize at the primary cilium membrane and basal body, enabling them to receive and transmit signals to the cell interior. Primary cilia are essential for the transduction of many signaling pathways, which is crucial for the brain development of such as Sonic Hedgehog (SHH), Wnt, MTOR, Notch, Hippo, PDGFR, or autophagy-related molecules (Wheway et al., 2018; Park et al., 2019). Among them, force sensing through regulation of primary cilia-SHH signaling gets much attention during brain development (Breunig et al., 2008; Liu et al., 2021). SHH signaling pathway is a master regulator of human brain development, contributing to patterning, cell proliferation and differentiation (Memi et al., 2018). SHH is secreted from the floor plate in the neural tube and acts as a morphogen to control cell identity in different regions of the developing brain (Marti et al., 1995; Roelink et al., 1995; Ye et al., 1998). Understanding these pathways is crucial for unraveling the mechanisms underlying mechanosensing and their impact on cellular behavior as well as their application to neural organoid generation.

Mechanical forces undergo alterations during brain development: neural tube formation (Sokol, 2016; Galea et al., 2017; Zhang et al., 2019), neural crest cell migration (Chevalier et al., 2016; Barriga et al., 2018), neural progenitor proliferation (Banerjee et al., 2009; Desmond et al., 2014; Petrik et al., 2018), neural progenitor differentiation (Keung et al., 2011; Pathak et al., 2014; Arulmoli et al., 2015; Rammensee et al., 2017; Nourse et al., 2022), neural/glial migration (Kengaku, 2018; Leclech et al., 2019; Minegishi and Inagaki, 2020; Nakazawa and Kengaku, 2020; Lopez-Mengual et al., 2022), axon outgrowth and guidance

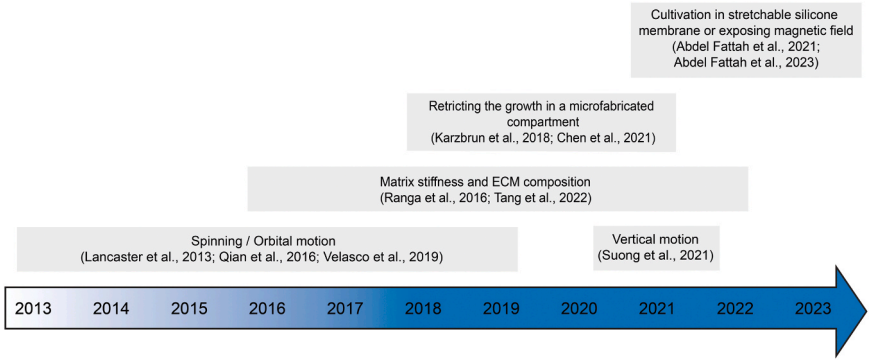

Fig. 1. Overview of human pluripotent stem cell-derived neural organoid generated by mechanical force application. Timeline for the development of neural organoids guided by mechanical forces with important milestones. Several engineering solutions have been developed to enhance the complexity, maturation, and function of neural organoids.

(Koser et al., 2016; Franze, 2020; Raffa, 2023), synapse formation and plasticity (Minegishi et al., 2023), and cortical growth and folding (Llinares-Benadero and Borrell, 2019; Del-Valle-Anton and Borrell, 2022). The cellular functions of the progenitors are also important in these areas.

3.2. Neural organoids guided by mechanical forces

For the need of clinical application, previous efforts were made to enhance the complexity and functionality of organoids, to develop techniques for their upscaling, and to improve long-term stability and develop standardized protocols. Among them, engineering by mechanical force is widely used, with such as bioreactors and microfluidics, to promote the growth and organization of neural organoids. Various types of bioreactors have been used for the long-term culture of neural organoids (Lancaster et al., 2013; Lancaster and Knoblich, 2014; Qian et al., 2016; Velasco et al., 2019; Suong et al., 2021) (Fig. 1). Bioreactors enable the improvement of oxygen and nutrition transfer, increase cell survival, and accelerate differentiation. Despite these advantages, it should be noted that these suspension culture systems might also form complex forces caused by fluid mixing on cells and organoids. These forces involve shear stress and turbulent energy that can affect cell viability and have a high impact on the differentiation and phenotype of organoids.

Previous studies revealed that fluid dynamics within bioreactors has a role in enhancing the pluripotency of pluripotent stem cells (Fridley et al., 2012). The nuclear translocation of β-catenin and interaction between β-catenin and adherens junction-associated vinculin are required for fluid shear stress-induced pluripotency (Nath et al., 2021). Different fluid forces depend on the agitation rate and size/shape of vessel impact spheroid formation such as aggregation size, homogeneity, and cellular metabolism as well as differentiation and function in comparison with static culture (Kinney et al., 2011).

Mechanical forces have significant influence on the formation and development of organoids (Hofer and Lutolf, 2021; Tortorella et al., 2022). Understanding and controlling the mechanical forces on organoids is essential for guiding their self-organization and morphogenesis, enabling the generation of more complex and functional organoid models. Different forces (shear stress, tension, compression, or hydrostatic pressure) and matrix properties (stiffness and elasticity) provide specific microenvironments for organoid formation and organization (Tortorella et al., 2022). Several studies have explored how forces influence organoid formation (Fig. 1). Restricting the growth of neural organoids in a microfabricated compartment enables the investigation of the biophysical forces that underlie the formation of brain folds (Karzbrun et al., 2018; Chen et al., 2021). Different matrix stiffness and ECM composition influence organoid formation, patterning, and organization (Ranga et al., 2016; Tang et al., 2022).

Moreover, extrinsic mechanical forces can guide the patterning of 3D organoids. Modulating the cytoskeleton structure by cultivating organoids in stretchable silicone membrane or exposing magnetic field to organoids enhances the growth and patterning of neural tubes (Abdel Fattah et al., 2021; Abdel Fattah et al., 2023). On the other hand, the effect of fluid flow on neural organoid formation has also been noticed. Continuous laminar flow increased dopaminergic differentiation of midbrain organoids (Berger et al., 2018), while bioreactors or orbital shakers are used to provide better nutrient and oxygen perfusion followed by better neural layer formation (Lancaster et al., 2013; Lancaster and Knoblich, 2014; Qian et al., 2016), or to increase reproducibility (Velasco et al., 2019).

3.3. Neural organoids generated by the mixing of vertical motion bioreactor

Mechanical forces generated by the mixing of vertical motion bioreactors had an impact on neural organoid specification (Suong et al., 2021). Computational analysis of fluid dynamic within vertical motion bioreactors showed that the agitation rate is proportional to the dynamic force characteristics such as flow velocity, shear stress, strain rate, vorticity, turbulent energy, and energy dissipation (Suong et al., 2021). The higher the mixing speed, the higher is the value of the fluid mechanics that neural organoids are subjected to. Surprisingly, even though vertical mixing provides lower flow velocity than orbital mixing, it allows for highly uniform dispersion of cells and the control of turbulent energy and energy dissipation. It was also observed that periodically high and low value of turbulent energy and energy dissipation due to amplitude oscillations provide a constant and non-steady stimulus to the entire cell surface. This suggested that the high amplitude of turbulent energy that occurs within a vertical motion bioreactor might be an induction factor for changing the neural organoid morphology. Organoids in the vertical motion bioreactor showed movement in radial and axial directions, whereas organoids in the orbital shaker just moved in a radial direction (Suong et al., 2021).

Cultivation of neural organoids in vertical motion bioreactor showed a dramatic change in their structure and identity (Suong et al., 2021). The morphology of neural organoids cultured in a vertical motion bioreactor had a different structure from that in an orbital shaker such as an inverted order of neurons/neuron progenitors and ventral organoid identity with the enrichment of GABAergic neurons. Further analysis found that primary cilia of neural progenitors showed a strong response to fluid dynamics in both quantity and direction. Neural progenitors in organoids generated using a vertical motion bioreactor exerted primary cilia in a random direction while those of control mainly showed an apical-basal direction. Moreover, activation of SHH pathway is also detected. These findings showed that controlling mechanical forces applied to neural organoids is one possible way to guide organoid

differentiation.

4. Future perspectives and challenges for the application of mechanical forces in neural organoid design

Over the past decade, the rapid development of neural organoid technology has enabled major applications to the investigation of the mechanisms of human brain development and disease. Subsequent efforts are going to focus on applying engineering solutions to overcome current limitations of the neural organoid field. The active application of mechanical forces of engineering in neural organoid design holds significant potential for advancing our understanding of brain development, modeling neurological diseases, and developing novel therapeutic strategies. Mechanical force engineering could allow researchers to create physiologically relevant environments for neural organoids, resulting in improvement of structural complexity and maturation. Furthermore, the study of how fluid dynamic forces affect neural organoid development could lead to insights into the fundamental mechanisms of brain growth, neuronal migration, and tissue organization. Optimizing the parameters of fluid flow within bioreactors will be necessary to find suitable culture conditions for each type of neural organoids.

In summary, despite the existing challenges, the ongoing development of mechanical force engineering in neural organoid design provides numerous advantages that will contribute to the application of organoids in a new era of human brain science.

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