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<td><strong>Author(s)</strong></td>
<td>Zhuang, Pei; An, Jia; Tan, Lay Poh; Chua, Chee Kai</td>
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THE CURRENT STATUS OF 3D BIOPRINTING FOR NEURAL TISSUE MODELS

PEI ZHUANG
Singapore Centre for 3D Printing, School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue
Singapore, 639798, Singapore

JIA AN
Singapore Centre for 3D Printing, School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue
Singapore, 639798, Singapore

LAY POH TAN
School of Materials Science and Engineering, Nanyang Technological University (NTU), 50 Nanyang Avenue, 639798, Singapore
Singapore, 639798, Singapore

CHEE KAI CHUA
Singapore Centre for 3D Printing, School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue
Singapore, 639798, Singapore

ABSTRACT: Neurological disease is one of the devastating diseases worldwide. Limited regeneration capability of central nervous system has greatly hindered the functional recovery of neural tissues. Bioprinting, offers a promising method to deposit cells, materials and instructive biomolecules in a precise arrangement simultaneously, which is possible to achieve the construction of multicellular functional neural tissues. In this review, we have 1) identified the essential design parameters of neural tissue constructs based on native tissue contexts 2) reviewed the updated studies of bioprinting for neural tissue applications 3) discussed the challenges and prospects of bioprinting for neural tissue development.

KEYWORDS: Bioprinting, Neural tissue, Tissue engineering, Biomaterials,

INTRODUCTION
Neurological diseases such as Parkinson's disease, Alzheimer's disease and traumatic brain injury have affected billions of people worldwide and even burden their families(Hopkins, DeSimone, Chwalek, & Kaplan, 2015). The poor regeneration capability of central nervous system poses great challenges to functional neural regeneration. This requires the suitable models that would enable deeper understanding of the pathology and development of such diseases. In the past decades, great efforts have been dedicated to generating in vitro 3D neural tissues models, including cell biology-based models (spheroids and organoids)(Dingle et al., 2015; Jo et al., 2016; Kato-Negishi, Morimoto, Onoe, & Takeuchi, 2013), and engineering-based models (scaffold and microfluidic platforms)(Tang-Schomer et al., 2014), which have been widely explored in terms of neural network mimicking, neurological diseases modelling and neurological
development (Zhuang, Sun, An, Chua, & Chew, 2018). Despite the great success that have been achieved with these models, their highly viable structures are inadequate to capture the cell-cell and cell-extracellular matrix (ECM) interactions. Bioprinting opens up a new avenue to construct neural tissue models in a more consistent manner owing to its superior capability of depositing cells and materials automatically and simultaneously.

This paper reviewed the up-to-date bioprinting studies on neural tissue applications. Design considerations for neural tissues are described in the first place. Followed by an overview of existing bioprinted neural models in terms of biomaterial and printing method. In the end, we discussed the limitations and potential in bioprinting for neural tissue applications.

**DESIGN PARAMETERS OF NEURAL TISSUES**

Some key factors need to be considered when designing *in vitro* neural tissue constructs. Firstly, multiple types of neurons and glial cells are found in different regions of the nervous system. In addition to neurons, glial cells such as oligodendrocytes, microglia, astrocytes and pericytes, should be considered in order to provide a full repertoire to replicate the nervous system.

Secondly, ECM is of critical importance in regulating the cell behaviors. Neural tissues possess very unique biophysical properties. As reported in (Benam et al.), neural tissue has extremely low mechanical stiffness (neonatal brain tissue 110 Pa and adult brain tissue <1 kPa) in comparison to other tissues such as bone, muscle, and heart. Materials that being adopted to mimic neural tissue should exhibit similar elastic modulus to support neuronal cells. Since most of the organs and tissues are formed via morphogen gradients, such factors are important and should be taken into account within printed tissues to shape the right 3D spatial orientation of cells.

In microscale, neural cells are very sensitive to topographical cues including microgrooves, pillars, pits and wrinkle. These have been proved to exert great effects on cell migration, differentiation and neurite outgrowth (Li, Katsanevakis, Liu, Zhang, & Wen, 2012). In macroscale, tissue structures play a critical role in presenting the cells with desired signals. Central nervous system mainly composed of brain and spinal cord. Brain is a highly compartmentalized structure with specific functions. For instance, the great loss of neurons in substantia nigra and striatum leads to Parkinson’s disease. The Alzheimer’s Disease damages the memorize ability and causes the shrinkage of hippocampus (D’Avanzo et al., 2015). Spinal cord is a long, tubular nerve bundle. Although they exhibit very distinct macro structures, some common features could be extracted from the microstructures. Such as layered brain cortex, neural networks, and frequently used aligned fibers mimicking spinal cord. These simplified function units may provide an insightful understanding into native tissue formation.

**PROGRESS IN BIOPRINTING OF NEURAL TISSUES**

Pioneer studies on neuronal cells (rat embryonic motor neurons) printing was conducted by Xu (Xu et al., 2006) though inkjet printing. Collagen was used as a substrate. The good survival rate of cells indicated that possibility of constructing neural tissues though inkjet printing. Since inkjet printing requires low viscosity materials, such other studies through inkjet printing all utilized biomaterials such as collagen, fibrin, polyacrylamide-based hydrogels or cell suspension (Christopher et al., 2016; Ilkhanizadeh, Teixeira, & Hermanson, 2007; Lee et al., 2010).
In comparison to droplet-based printing (inkjet printing) which is applicable for materials with low viscosities ranging from 3.5 to 70 mPa s, while extrusion-based bioprinting is able to print materials with higher viscosities (Murphy & Atala, 2014). Alginate, gelMA, chitosan, agarose, polyurethane, gellan gum have been reported as promising bioinks for neuronal cells printing (Gu et al., 2016; Gu, Tomaskovic-Crook, Wallace, & Crook, 2017; Hsieh, Lin, & Hsu, 2015; Lozano et al., 2015; Wei, Harris, & Zhang, 2016; Zhou et al., 2018).

Apart from printing cell-material mixture, scaffold printing for neural tissue regeneration has made some progress as well. Johnson et al created a customized nerve graft bifurcating sensory and motor nerve pathways using a combination of 3D imaging and microexclusion printing techniques (Johnson et al., 2015). A 3D model was first reverse engineered via scanning of human patient using structured light scanning (SLS) technique. Next 3D printing following this model produced a fully personalized scaffold, which imitate various physical and structural parameters of the original nerves with a high degree of fidelity. The author then used this scaffold to investigate factors that promote endogenous nerve regeneration. Given previous studies that have implicated physical cues in neurite arborization, they fabricated microgrooves within their scaffold and found them to exert a profound influence on neurite alignment. The effect was consistently observed among different primary neurons tested, including superior cervical ganglia (SCG), dorsal root ganglia (DRG), and Schwann cells. In addition to physical cues, the authors also examined the effect of biochemical cues of nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF), and found them to be instructive in directing sensory and motor nerve branches to grow separately along specific paths. To extend these in vitro findings to animals, they created 10 mm gaps in the sciatic nerve of rats and implanted the animals with personalized 3D printed nerve guides. Over a 3-month course, the walking of the rats was significantly improved, suggestive of functional regeneration. Additional analysis confirmed cellular recovery including enhanced neurite innervations. Thus, precision 3D scaffold printing, enabled by accurate 3D geometric rendering, and incorporation of physical as well as biochemical guidance cues, represent a promising method for complex nerve regeneration.

Table 1. Bioinks for construction of 3D neural tissue models.

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<tr>
<th>Bio-ink</th>
<th>Printing Method</th>
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<tr>
<td>Collagen</td>
<td>Inkjet printing</td>
<td>(Xu et al., 2006)</td>
</tr>
<tr>
<td>Fibrin</td>
<td>Inkjet printing</td>
<td>(Lee et al., 2010)</td>
</tr>
<tr>
<td>Polyacrylamide-based hydrogels</td>
<td>Inkjet printing</td>
<td>(Ilkhanizadeh et al., 2007)</td>
</tr>
<tr>
<td>Cell suspension</td>
<td>Inkjet printing</td>
<td>(Christopher et al., 2016)</td>
</tr>
<tr>
<td>Polyurethane/graphene</td>
<td>Extrusion-based printing</td>
<td>(Huang, Kumar Shrestha, Ariga, &amp; Hsu, 2017)</td>
</tr>
<tr>
<td>GelMA-DA</td>
<td>Extrusion-based printing</td>
<td>(Zhou et al., 2018)</td>
</tr>
<tr>
<td>GelMA/graphene</td>
<td>Extrusion-based printing</td>
<td>(Wei et al., 2016)</td>
</tr>
<tr>
<td>alginate/chitosan/agarose/carboxymethyl</td>
<td>Extrusion-based printing</td>
<td>(Gu et al., 2016; Gu et al., 2017)</td>
</tr>
<tr>
<td>Polyurethane</td>
<td>Extrusion-based printing</td>
<td>(Hsieh et al., 2015)</td>
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CURRENT LIMITATIONS AND POSSIBILITIES
Although bioprinting has made some notable progress in other tissue models, such as skin, muscle and bone, its applications in neural tissue still limited (as shown in Table 1). Critical challenges remain to be addressed.

Incorporation of multiple cell types
Despite some studies have printed multiple cell types, only limited neural cell types have been printed. Glial cells should be incorporated to fully recapitulate the cell-cell and cell-ECM interactions. However, this will further require the knowledge of co-culture and the materials that can better support the viability and functional integrity of various cell types. Stem cells are more attractive due to its intrinsic ability to differentiate into diverse cell types. This offers an alternative to tissue model construction. Through the assist of appropriate differentiation cues, bioprinting of stem cells may allow the construction of artificial neural tissues with more complex cellular arrangement.

The conflict between printing process and tissue modulus
Due to the weak mechanical property of neural tissues, it poses great challenges in finding a material that matches the mechanical stiffness of neural tissues and yet, has a superior printability to maintain the structure fidelity after several layers of material deposition. Existing printing strategies have offered some promising alternatives. Such as printing in a supportive bath which is possible to support the weak materials. Additionally, hydrogels with dynamic mechanical property is of great interest. Bioinks transited from stiff to soft yield highly desirable property that is initially bioprintable and subsequently cell-responsive

Vascularization
In vitro culture system supporting long term viability and functionality of tissue models. High cell density will probably induce the necrosis in the central zone of the models, particularly for large constructs, which calls for proper vascularization. Thus, it is important to integrate some capillary networks in the tissue to facilitate the gas and nutrient exchange. In addition, various biomolecules required for controlling proper cell fate determination, structural organizations, and functional connections would need to be incorporated into the tissue models in a spatially and temporally regulated manner.

CONCLUSION
The mind-boggling structures of neural tissues pose great challenge to recapitulate the cell-cell and cell-ECM interactions. Bioprinting enables the manipulation of droplet to deposit cells and materials in a more systematic and consistent manner, which highlights the great potential in reconstructing functional neural tissue models. However, bioprinting of neural tissues is still in initial stage. Further advances such as bioink development, corporation of multiple cell types, optimal structural design, increased printing resolution will likely to be made to consolidate the existing models with higher fidelity.
Acknowledgments

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References


