PERSPECTIVE

Engineering nerve guidance conduits with three-dimenisonal bioprinting technology for long gap peripheral nerve regeneration

Nerve guidance conduits (NGCs) are tubular structures that are used to bridge the gap of a severed nerve, thereby acting as a guide and protective micro-environment for the regenerating axons and as a barrier against the in-growth of scar-forming tissue. In the last few decades, the concept of NGCs has evolved from a research tool to investigate nerve regeneration into a translational product that is now being used clinically as an alternative for autologous nerve graft repair, due to their availability and ease of fabrication. At the moment, various nerve conduits have been approved for clinical use and are being marketed, including Neurotube™ (K983007, 1999), Salubridge™ (K002098, 2000), NeuraGen™ (K011168, 2001), Surgisis™ Nerve Cuff (K031069, 2003), Neurolac™ (K050573, 2005; K112267, 2011), Cova™ ORTHO-NERVE (K103081, 2012), Reaxon™ Plus (K143711, 2015) (Du et al., 2018a). However, clinical outcomes associated with the use of artificial nerve conduits are often inferior to that of autografts, particularly over long lesion gaps. Although their clinical use has been limited, mainly to the repair of relatively small defects (< 3 cm), such as small-caliber digital nerves, the potential for extending their clinical application to the repair of larger defects and larger mixed or motor nerves has made the development of an ideal nerve tube appealing for both scientists and the medical device industry.

The NGCs can be made with impermeable, semi-permeable, or fully permeable outer tube walls that control access of nutrients/oxygen, growth factors, waste products, revascularization, and scar-producing fibroblasts in a radial direction between the site of injury and the surrounding microenvironment. This variability in permeability has increasingly been identified as a key mediator of NGC success. Of these, semipermeable conduits were found more suitable as they facilitate mass transport, vascular network formation, and Schwann cell migration, and inhibit fibroblast cell infiltration. Small rodent studies of the effect of conduit pore size on the outcome of nerve regeneration with conduits less than 3 cm have shown the optimal pore size to enable nutrient and waste diffusion and minimize fibrotic and inflammatory cell infiltration is in the range of 5–30 μm (Clements et al., 2016). As longer conduits are required in more severe nerve injuries, a greater porosity might be necessary to overcome limitations of nutrient and waste exchange.

In order to enhance the suboptimal performance of hollow tubular constructs, particularly on large gap repair, it is necessary to investigate the use of filled NGCs whereby the hollow luminal space of tubular constructs is replaced by additional supporting biomaterial. For example, one of the simplest structural modifications is to introduce a hydrogel-based luminal filler (Du et al., 2018b). When porous conduits are filled with a hydrogel, a balance must be achieved between the conduit porosity and the bioactivity of the filler matrix to encourage faster axonal regeneration than fibrotic tissue infiltration. Degradation rates of the hydrogel are also the factors in determining the suitability of the NGCs *in vivo*, as rapid degradation is associated with loss of structure and failure of the scaffold and degrading too slowly is associated with cell death and fibrosis. The significant success obtained by introducing interior topography to promote peripheral nerve regeneration has led researchers to further explore the organization of interior fillers. It has now become widely accepted that the microscale architectural guidance has been identified as a key parameter in the regenerative process due to the linear and nonlinear mechanics, which are crucial factors in cellular functions, intracellular communication, and tissue protection. Biological gels that are made of fibrous network architecture give rise to unique properties, like low concentration, high porosity gels with high mechanical responsiveness as a result of strain-stiffening, as well as the controllable architecture.

Incorporation of different fibrous filler materials within the NGC has been reported to facilitate axon regeneration. These methods have included using a macro- or nano-fiber matrix, multilumen/multichannel structures, and magnetically aligned fibrils. Conduits with micro- and nanostructure have been used in the biomimetic design and showed success bridging up to 30 mm sciatic nerve gaps in rats (Biazar and Keshel, 2013). The presence of micro/nano-scale alignment in the proximo-distal direction within the architecture of tubular constructs can enhance the peripheral nerve repair at the cellular level by increasing the efficiency of Schwann cell migration and axonal pathfinding, resulting in improved motor functional recovery (Du et al., 2017) and muscle mass and isometric force recovery (Jin et al., 2012). However, control over the complexity in the inner architecture of tubular constructs has historically been limited by the available biofabrication techniques, mimicking such three-dimensional (3D) complexity and the multicellular interactions naturally occurring in nerve structures represents one of the greatest challenges in nerve regeneration.

Recently developed 3D bioprinting technology, including droplet-, extrusion- and laser-based bioprinting, has been proven as a promising alternative to address some of these key challenges (Zhang et al., 2019). In nature, nerve tissues are organized with complex heterotypic 3D architectures, specific cell-cell interactions, anisotropic mechanical properties, and heterogeneous distribution of growth factors. 3D bioprinting enables the precise positioning of cells and biomaterials in 3D with finely tuned internal and external architectures, while being customizable to patient-specific needs (Johnson et al., 2015). Moreover, it allows for on-demand and scalable fabrication of complex designs, while being compatible with various scaffold materials and cell sources To gain desirable biocompatibility and superior mechanical properties, hybrids and composites that combine natural materials with synthetic polymers are the better option, which offer the technical possibility that chemical and physical properties (*e.g.*, porosity, surface characteristics, and degradation products nature) can be specifically optimized for a particular application. 3D printing also enables the controlled distribution of pores and manipulation of pore size throughout the material matrix of a printed object by the printer software setting for infill percentage, or the percentage of the printed object's volume occupied with the material.

While still in its early stages, bioprinting strategies have demonstrated their potential use in nerve regeneration to generate a variety of transplantable NGCs. 3D printing makes possible the reproduction of complex nerve features, such as branching nerve networks and intrinsic chemical mechanisms that steer motor and sensory axons along correct anatomical pathways (Johnson et al., 2015). A variety of NGC designs, varying in complexity and size, have been created including a life-size biomimetic branched human facial NGC (Zhu et al., 2018). *In vivo* implantation of NGCs with microchannels into a critical size defect (15 mm) in the rat sciatic nerve injury model demonstrated that the regeneration potential was equivalent to that of autografts in terms of nerve physiology, morphology, as well as cellular and structural aspects.

Moreover, various living cell types with scaffolds have been utilized together with 3D bioprinting technique to support nerve regeneration including Schwann cells, olfactory ensheathing cells, mesenchymal stem cells, and neural stem cells. Living cells can be either seeded into scaffolds or embedded into printing media during the scaffold-manufacturing process. The gelatin methacryloyl gel impregnated with adipose-derived stem cells nerve graft has been 3D printed into NGCs and shown the contribution of sciatic nerve functional recovery and axonal regeneration, which is approaching and exceeding those observed from autografts and acellular gel conduits (Hu et al., 2016). Schwann cells have been 3D bioprinted with composite hydrogels of alginate, fibrin, hyaluronic acid, and arginylglycylaspartic acid peptide to promote the alignment of Schwann cells inside scaffolds and thus provide haptotactic cues for nerve regeneration (Ning et al., 2018).

Overall, as nerve tissues comprise a complex, anisotropic, and hierarchical structure, regenerative matrices have to provide the

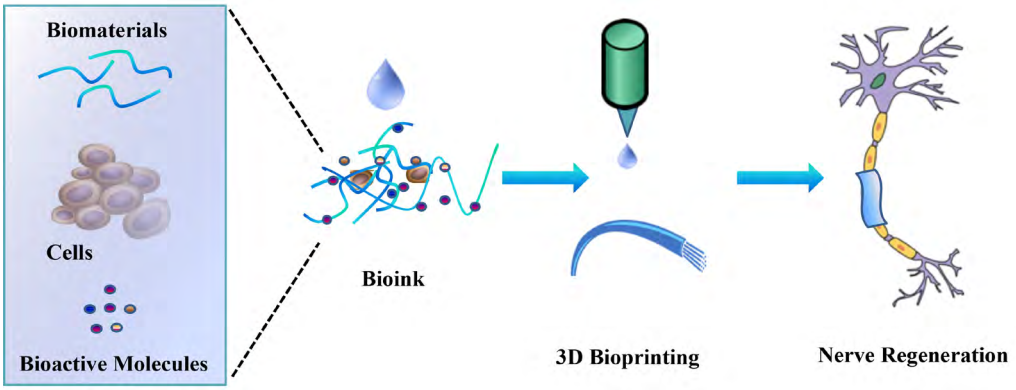

Figure 1 Three-dimensional (3D) bioprinting strategy for peripheral nerve regeneration.

correct architecture to guide cell organization during the healing process. 3D printing technologies now have the opportunity to improve upon the autograft and regenerate large gap injuries that are currently resistant to full recoveries. Bioprinting enables the production of NGC with the precise placement of biomaterials, transplanted cells, neurotrophic factors, and other biomolecules into spatially predefined locations (**Figure 1**). The 3D printed conduit could contain enough structural details that are just sufficient to facilitate nerve regeneration and integration of the conduit matrix into the existing nerve network, and to resist stresses encountered during normal bodily movements. Despite great promise in printing NGCs, current bioprinting approaches still have technical challenges precluding the formation of the desired nerve construction with an appropriate level of structural detail, high-resolution multiple cell types deposition, controlled cell distributions, vascularization, and innervation within complex 3D tissues. A proof-of-concept, reprogrammable NGC with multiresponsive architecture has been demonstrated with human mesenchymal stem cells by using stereolithography-based 4D printing (Miao et al., 2018). Organ-on-a-chip has also shown promising results. It provides the ability for testing multiple cell types without requiring complex models or living systems. Research investigating *in situ* printing into defect sites is another promising development of bioprinting; yet, this area of research still has many bridges to cross before entering the clinical world. Printing in situ will avoid the time-consuming stage of scaffold preparation and cell *in vitro* manipulations, eliminate any risks of contamination during post-fabrication, and avoid adverse effects induced by sterilization post-fabrication, such as changes to morphology and mechanical integrity of the scaffolds. Overcoming the biological, technological, and regulatory challenges to advance this 3D printing NGCs to widespread clinical use will only be possible through an integrated approach with a combination of technologies from the fields of biomaterial science, engineering, cell biology, and reconstructive microsurgery.

This work was partially supported by Maryland Stem Cell Research Fund (2018-MSCRFD-4271, to XJ), and R01HL118084 and R01NS110387 from United States National Institutes of Health (both to XJ).

Jian Du, Xiaofeng Jia*
Department of Neurosurgery, University of Maryland School of Medicine, Baltimore, MD, USA (Du J, Jia X)
Department of Orthopedics, Anatomy Neurobiology, University of Maryland School of Medicine, Baltimore, MD, USA (Jia X)
Department of Biomedical Engineering, Anesthesiology and Critical Care Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA (Jia X)
***Correspondence to:** Xiaofeng Jia, MD, PhD, xjia@som.umaryland.edu.*
***orcid:** 0000-0003-1445-8525 (Xiaofeng Jia)*
***Received:** May 4, 2019*
***Accepted:** May 31, 2019*
***doi:** 10.4103/1673-5374.262580*

***Open peer reviewer:** Sean I. Patterson, Universidad Nacional de Cuyo, Argentina.*

References

Biazar E, Keshel SH (2013) Chitosan-cross-linked nanofibrous PHBV nerve guide for rat sciatic nerve regeneration across a defect bridge. ASAIO J 59:651-659.
Clements BA, Bushman J, Murthy NS, Ezra M, Pastore CM, Kohn J (2016) Design of barrier coatings on kink-resistant peripheral nerve conduits. J Tissue Eng 7:2041731416629471.
Du J, Chen H, Qing L, Yang X, Jia X (2018a) Biomimetic neural scaffolds: a crucial step towards optimal peripheral nerve regeneration. Biomater Sci 6:1299-1311.
Du J, Liu J, Yao S, Mao H, Peng J, Sun X, Cao Z, Yang Y, Xiao B, Wang Y, Tang P, Wang X (2017) Prompt peripheral nerve regeneration induced by a hierarchically aligned fibrin nanofiber hydrogel. Acta Biomater 55:296-309.
Du J, Zhen G, Chen H, Zhang S, Qing L, Yang X, Lee G, Mao HQ, Jia X (2018b) Optimal electrical stimulation boosts stem cell therapy in nerve regeneration. Biomaterials 181:347-359.
Hu Y, Wu Y, Gou Z, Tao J, Zhang J, Liu Q, Kang T, Jiang S, Huang S, He J, Chen S, Du Y, Gou M (2016) 3D-engineering of cellularized conduits for peripheral nerve regeneration. Sci Rep 6:32184.
Jin J, Park M, Rengarajan A, Zhang Q, Limburg S, Joshi SK, Patel S, Kim HT, Kuo AC (2012) Functional motor recovery after peripheral nerve repair with an aligned nanofiber tubular conduit in a rat model. Regen Med 7:799-806.
Johnson BN, Lancaster KZ, Zhen G, He J, Gupta MK, Kong YL, Engel E, Krick KD, Ju A, Meng F, Enquist LW, Jia X, McAlpine MC (2015) 3D printed anatomical nerve regeneration pathways. Adv Funct Mater 25:6205-6217.
Miao S, Cui H, Nowicki M, Xia L, Zhou X, Lee SJ, Zhu W, Sarkar K, Zhang Z, Zhang LG (2018) Stereolithographic 4D bioprinting of multiresponsive architectures for neural engineering. Adv Biosyst doi: 10.1002/adbi.201800101.
Ning L, Sun H, Lelong T, Guilloteau R, Zhu N, Schreyer DJ, Chen X (2018) 3D bioprinting of scaffolds with living Schwann cells for potential nerve tissue engineering applications. Biofabrication 10:035014.
Zhang L, Yang G, Johnson BN, Jia X (2019) Three-dimensional (3D) printed scaffold and material selection for bone repair. Acta Biomater 84:16-33.
Zhu W, Tringale KR, Woller SA, You S, Johnson S, Shen H, Schimelman J, Whitney M, Steinauer J, Xu W, Yaksh TL, Nguyen QT, Chen S (2018) Rapid continuous 3D printing of customizable peripheral nerve guidance conduits. Mater Today (Kidlington) 21:951-959.

P-Reviewer: Patterson SI; C-Editors: Zhao M, Li JY; T-Editor: Jia Y