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3D-printed nerve conduit with vascular networks to promote peripheral nerve regeneration

Jie Tao[a,b,1], Yan He[b,1], Shuai Wang[b], Jian Mao[a,*]

[a] School of Materials Science and Engineering, Sichuan University, Chengdu, Sichuan 610065, China
[b] Department of Biotherapy, Cancer Center, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, Sichuan 610065, China

A B S T R A C T

Peripheral nerve regeneration remains a challenge in tissue engineering and regenerative medicine. However, the existing approaches have limited regenerative capability. 3D-printed nerve conduits with well-defined properties are potent tools to facilitate peripheral nerve regeneration after injuries. Meanwhile, the vascular networks within the constructs can promote the exchange of oxygen, neurotrophic factors, and removal of waste products, thereby providing an advantageous microenvironment for tissue regeneration. It will be an interesting approach to integrate 3D-printed nerve conduit with vascular networks for the guidance of regenerated nerves. We hypothesize that 3D-printed vascularized nerve conduit will be an effective platform to promote nerve regeneration and functional restoration.

Introduction

Peripheral nerve injuries commonly impair the ability to move and feel, causing greater than 200 000 individuals in disability annually in USA [1,2]. To treat the gaps (> 5 mm) in peripheral nerves, autografts remain the gold standard [3]. However, the inherent limitations restrict their wide clinical use, including donor site morbidity, need of additional surgery, and neuroma formation at the surgical sites [4]. To address these challenges, artificial nerve conduits consisted of natural or synthesized biomaterials have been proposed for guiding the axonal elongation and cells migration, leading to the peripheral nerve regeneration and functional restoration [5]. Although numbers of strategies have been proposed to offer a better microenvironment (e.g., neurotrophic factors [34], Schwann cells [35], stem cells [36], and multi-channels [37]) for functional restoration, the efficacy is still unsatisfying. Only partial functions can be restored, leading to a great need for the fabrication of advanced nerve conduits. Recently, biomimetic structure of the implanted scaffolds has been found to promote tissue regeneration, which inspires the fabrication of nerve conduits with biomimetic structure [6–8]. The biomimetic nerve conduits might provide a promising strategy for peripheral nerve regeneration.

3D printing technology with the ability to fabricate complex and customized structure is emerging as a powerful tool in various biomedical applications [9,10]. Based on the personalized data, different biomaterials and cells can be installed in a highly precise control and provide a tissue-mimicking microenvironment by 3D printing technology, leading to tissue regeneration after injury. Recently, on the front of 3D printing technology, 3D-printed scaffolds are increasingly combined with agents with functional roles (e.g. cells, nanoparticles, growth factors, and bacteria) for advanced therapy [11–13]. We previously took advantage of digital light processing (DLP)-based 3D bioprinting technology and functional nanoparticles to construct functional nerve conduits [14,15]. In these studies, we found that some of the hydrophobic drugs show abilities in promoting nerve regeneration after injuries. Yet, direct administration of these hydrophobic drugs might cause side effects. We took advantages of nanotechnology to incorporate the drug into the nanoparticles, followed by mixing with the polymers for bioprinting. The prepared nerve conduits could only provide channels for axonal elongation, but also sustained-release the regenerative drug for the migration and remyelination of Schwann cells. Herein, 3D printed scaffolds with functional roles and biomimetic structure allow a promising strategy for peripheral nerve regeneration.

The hypothesis

The vascularization in implanted biomaterials supplies the growth and trophic factors for cells' proliferation and migration, while preventing the atrophy of distal tissues. 3D printing technology allows the precise design and fabrication of customized constructs with vascular networks. It will be a great potential to fabricate constructs with vascular networks for tissue regeneration by 3D printing technology. Therefore, we hypothesize that 3D-printed vascularized nerve conduits

* Corresponding author.
E-mail address: maojian@scu.edu.cn (J. Mao).
[1] These authors contributed equally to this work.

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will be an effective platform for axonal elongation and remyelination. We can design the nerve conduits and the vascular networks from the magnetic resonance imaging (MRI)/computer tomography (CT) data of the patients. The customized nerve conduits will be highly precise-matched to the transected nerves for promoting nerve regeneration.

The theory of our hypotheses

Despite the certain regenerative capability in the peripheral nerve system (PNS), it is still a crucial challenge to repair the long defects in clinic and the functional restoration is usually unsatisfying [16]. A series of pathophysiological events occur after peripheral nerve injury [17], a Wallerian degeneration will occur within 96 h in the distal sites [18]. Schwann cells proliferate and change their phenotype from a role supporting normal nerve transmission to function supporting regeneration [19], while the macrophages are recruited to remove the degradative products [20]. Next, axons sprout from the nodes of Ranvier in the proximal site, and undergo remyelination *via* Schwann cells until reaching the targeted tissues or organs [21]. Both cellular and molecular events distal to nerve injury lead the development of a microenvironment that stimulates axonal regrowth from proximal stumps and supports axonal regeneration. However, the transected peripheral nerves without treatment cannot be re-connected and establish biological signals to the targeted tissues/organs. Artificial nerve conduits are the promising methods to provide the physical channels, which guide the extension of axons [22,23]. During the past decades, there are a number of nerve conduits that have been approved by Food and Drug Administration (FDA) and used in clinic [24].

Currently, several fabrication methods are proposed to prepare nerve conduits, such as electrospinning [25], molding [26], and dip-coating technique [27]. Nonetheless, these methods offer limited control in dimensions and low precision. Three-dimensional (3D) printing technology provides an advanced fabrication method for constructing personalized and complex nerve conduits [28]. Recently, taking advantage of 3D printing technologies to fabricate nerve conduits has become a compelling issue in tissue engineering [29–31], demonstrating the potential of using 3D printing technology to construct nerve conduits. The 3D printing technologies allow the feasibility to fabricate conduits with complex structure and designed functions. Meanwhile, 3D printing technology can not only enable the fabrication of nerve conduits with customized structure, but also offer the additional engineering flexibility to prepared vascular networks [32,33], especially for preparing capillaries in peripheral nerves. Multi-biomaterials and cells can be programmed installed in the personalized biomimetic scaffolds to form endothelialized networks *via* 3D printing technology. The prepared constructs with vascular networks display capability in inducing therapeutic angiogenesis [34]. For instance, Arakawa, Christopher K et al. took advantages of a photodegradable biomaterial and 3D bioprinting technology to generate endothelialized networks [35]. 3D printing technology presents the capabilities to construct a nerve conduit containing endothelialized vascular networks. The nerve conduits could be prepared through the following steps. Firstly, we can take advantages of MRI or CT data of the patients to design and fabricate the nerve conduits through 3D printing technology. Then, the endothelial cells can be injected into the vascular networks to form the connective vessels [34].

The 3D-printed nerve conduits induce the regeneration of the injured nerves from the axons-guided channels and the vascular networks. The mechanisms of the channels that guide the regeneration of nerves have been introduced by previous studies [5,36]. To date, there is no study reporting the nerve conduits with vascular networks. The vascular networks within the conduit might enhance the efficacy from the following aspects. Firstly, the prepared vascular networks can facilitate nutrition delivery for nutrient diffusion and ensure temperature, pH, oxygen, glucose, and salt homeostasis, which do the favor for the axonal elongation and remyelination of Schwann cells. Next, due to the transection of the nerves, partial supply of nutrition to the targeted muscles is cut off, which would result in the atrophy of the targeted muscles. The rapid formation of vascular networks can reduce the atrophy of the targeted muscles. Recently, Cattin Anne Laure et al. have found that blood vessels directed the migrating cords of Schwann cells, leading to peripheral nerve regeneration after injury [37]. This result demonstrated the importance of vessels in peripheral nerve regeneration and functional restoration after nerve injuries. Based on the mentioned information above, the combination of vascularity and artificial nerve conduits will provide a promising approach for nerve repair. Therefore, it will be a potent strategy to fabricate a conduit with vascular networks for the peripheral nerve regeneration.

Conclusion

To sum up the above-mentioned theories, we could prepare nerve conduit with vascular network for bridging the gaps in peripheral nerves *via* 3D printing technology. 3D printing technology allows for the design and fabrication of personalized nerve conduits with high spatial control. Meanwhile, the endothelialized vascular networks can be precisely installed within the nerve conduits. These conduits provide a favorable microenvironment for axonal extension and remyelination, while the endothelialized vascular networks facilitate the proliferation and migration of cells, and prevent the atrophy of muscles and loss of function. The proposed hypothesis provides a novel and promising strategy to design and fabricate enhanced nerve conduits to promote peripheral nerve regeneration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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