Burns & Trauma, 2021, 9, tkab011
https://doi.org/10.1093/burnst/tkab011
Review

Review

3D printing of functional nerve guide conduits

Yulan Huang[1], Wenbi Wu[1], Haofan Liu[1], Yuwen Chen[1], Bo Li[1], Zhiyuan Gou[1], Xun Li[1,2] and Maling Gou[1,*]

[1]State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu, 610041, China and [2]Department of Ophthalmology, West China Hospital, Sichuan University, Chengdu, 610041, China

*Correspondence Email: goumaling@scu.edu.cn

Received 6 December 2020; Revised 24 January 2021; Editorial decision 22 March 2021

Abstract

Nerve guide conduits (NGCs), as alternatives to nerve autografts and allografts, have been widely explored as an advanced tool for the treatment of peripheral nerve injury. However, the repairing efficiency of NGCs still needs significant improvements. Functional NGCs that provide a more favorable microenvironment for promoting axonal elongation and myelination are of great importance. In recent years, 3D printing technologies have been widely applied in the fabrication of customized and complex constructs, exhibiting great potential for tissue engineering applications, especially for the construction of functional NGCs. In this review, we introduce the 3D printing technologies for manufacturing functional NGCs, including inkjet printing, extrusion printing, stereolithography-based printing and indirect printing. Further, we summarize the current methods and strategies for constructing functional NGCs, such as designing special conduit architectures, using appropriate materials and co-printing with different biological cues. Finally, the challenges and prospects for construction of functional NGCs are also presented.

Key words: Nerve guide conduits, Functionalization, Peripheral nerve repair, 3D printing

Background

Peripheral nerve injury (PNI) is one of the most common neuropathies, mainly caused by trauma, accident and disease [1,2]. PNI often impairs the motor and sensory abilities of patients and results in paralysis and disability, which may significantly reduce the quality of a patient's life [3]. The self-repairing ability of peripheral nerves is extremely limited and treating PNI remains challenges in regenerative medicine [4]. Currently, autografts are regarded as the gold standard to repair PNI. However, there are also some disadvantages of autografts, such as the need for sophisticated surgeries, neuroma formation, lack of nerve donors and donor-site morbidity [5–7]. Hence, exploring alternative therapy is of vital significance. Nerve guide conduits (NGCs), as exogenous alternatives to bridge the injured nerve stumps, can build a beneficial microenvironment for promoting nerve recovery by complex mechanisms [8]. So far, some single-channel NGCs, such as Neuromaix®, NeuraGen® and Reaxon Plus® [9,10], have been approved by the Food and Drug Administration (FDA) for PNI treatment. However, the curative effect of normal NGCs in the therapy of long-gap PNI is not very satisfactory and still needs much improvement.

Functional NGCs can provide a favorable microenvironment, which is useful when treating PNI and beneficial to the functional recovery of the injured nerve [11–13]. To develop functional NGCs, some physico-chemical cues and biological cues are introduced to promote axonal elongation and myelin sheath formation. On the one hand, the customized structures of NGCs can provide biomimetic physical support to facilitate nerve regeneration. On the other hand, the material composition of NGCs and the corresponding physical and chemical properties may affect cellular behaviors and further

© The Author(s) 2021. Published by Oxford University Press.
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022

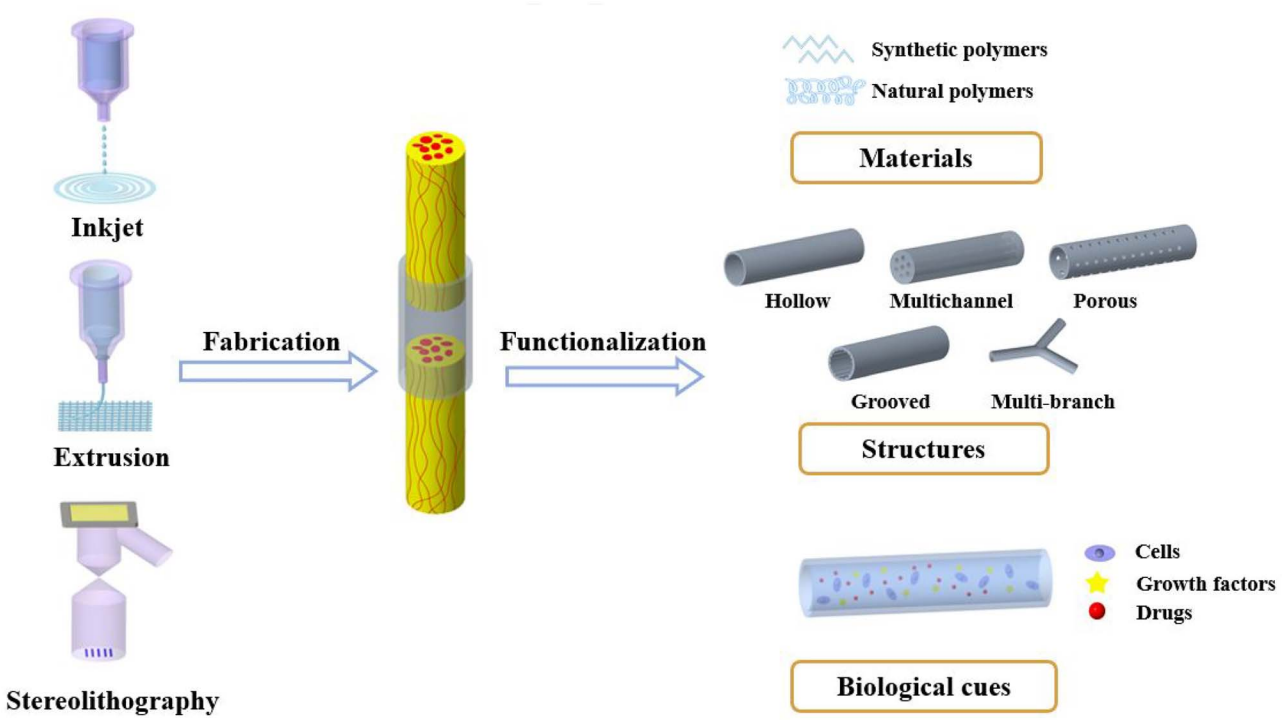


Figure 1. Schematic diagram of 3D printing technologies for NGCs fabrication and the strategies for construction of functional NGCs. *NGCs* never guide conduits

influence the efficiency of nerve recovery. For the biological cues, some biological elements such as cells, neurotrophic factors and drugs are also integrated with NGCs to enhance the repair efficiency.

Recently, the newly emerging 3D printing which can construct complex structures rapidly has been applied to fabricate many meaningful products, such as personalized vascular topologies, cardiac patches and hearts [14–19], exhibiting great potential applications in tissue engineering. 3D printing can not only fabricate flexible NGCs with sophisticated structures, but also combine the scaffolds with cells and growth factors to mimic the extracellular matrix [20]. Moreover, 3D printing is a highly flexible processing technique with high precision [21,22] and thus is superior for the construction of functional NGCs for PNI repair.

Herein, we introduce the current technologies for 3D printing NGCs based on different principles and summarize the current methods and strategies of preparing functional NGCs, including designing conduit structures and materials, and co-printing with different biological cues (Figure 1). Finally, the challenges and prospects for the construction of functional NGCs are discussed.

Review

3D printing technologies for NGCs

Different from the traditional fabrication methods of NGCs, such as solvent casting, freeze drying and electrospinning [23,24], 3D printing technology can fabricate NGCs with personalized characteristics to imitate the natural structure of nerves. NGCs can be created layer-by-layer by rapid prototyping machines, which is assisted by computer-aided design (CAD) technology by collecting and digitizing the complex microarchitectural information of native tissue from the images acquired by computed tomography (CT) or magnetic resonance imaging (MRI) [25–28]. In general, extrusion, inkjet and stereolithography printing are the most widely used 3D printing methods to fabricate NGCs. Moreover, indirect 3D printing is another way to construct different structures for NGCs using printed customized molds. The characteristics of different 3D printing technologies for NGCs are summarized in Table 1.

Inkjet 3D printing Inkjet printing is also known as drop-on-demand printing. The bioink drop can be artificially controlled and delivered to predefined locations by thermal or piezoelectric forces while printing. The resolution of inkjet printing can range from 1.0 to 500 μm depending on the bioink and sizes of nozzles [29,30]. A cylindrical NGC composed of polylactic acid (PLA) and poly(ε-caprolactone) (PCL) was constructed by a piezoelectric inkjet system with a resolution of 1.0 μm [31]. Another study has shown that neuronal and Schwan cells can keep normal cell proliferation and viability when printed with a 60 μm single-nozzle piezoelectric inkjet device, indicating that this technique can be utilized for bioprinting application [32].

Extrusion 3D printing Different from the drop-by-drop of inkjet printing, extrusion printers fabricate a 3D scaffold

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022

Table 1. Different kinds of 3D printing technologies for NGCs

Technology	Materials	Advantages	Disadvantages	Reference
Inkjet	PLA/PCL, collagen	1. High speed 2. Low cost	1. Low mechanical strength 2. Bioprinting limited. Unable to control the directionality and size of droplets precisely	[29,31,32,50]
Extrusion	PLLA, PLGA, GelMA, alginate dialdehyde etc.	1. Low cost 2. Multiple materials mixed printing 3. Higher cell viability	1. Lower resolution than inkjet 2. Low speed 3. Nozzle clogging	[35,37–39]
Stereolithography	Photopolymerizable monomer like GelMA, PEGDA, silk-GMA, glycerol sebacate methacrylate and composites	1. Higher resolution than extrusion 2. High fabrication speed 3. Personalization	1. Expensive 2. Photocurable resin limited 3. Possible cytotoxicity of residual photoinitiator and uncured resin	[41,44,51–54]
Indirect	GelMA, alginate methacrylate etc.	1. Convenience 2. Low cost	1. Microstructures limited 2. Personalisation	[46–49,55]

PLA/PCL polylactic acid and poly(ε-caprolactone), *PLLA* poly (L-lactic acid), *PLGA* poly (lactic-co-glycolic acid), *GelMA* gelatin methacrylate, *PEGDA* polyethylene glycol diacrylate, *silk-GMA* silk fibroin-glycidylmethacrylate, *NGCs* never guide conduits

line-by-line with a movable nozzle driven by pneumatic or mechanical dispensing systems, with a resolution ranging from 100 to 500 μm [33,34]. Johnson *et al*. developed a custom 3D printing system based on microextrusion and structured light scanning (SLS) technique, and 3D-printed silicone bifurcated NGCs, which significantly enhanced the functional recovery of a 10 mm-defect of a rat sciatic nerve bifurcation [35]. In another study, uniaxial and multichannel conductive NGCs were pressure-extruded with a minimal 100 μm tip. These conduits with excellent physical and mechanical performances could be sutured with a 2 cm nerve defect from the dorsal branches of the ulnar nerve in an unfixed human cadaver by complex surgical process [36]. Besides, through bioprinting technology, different human umbilical vein endothelial cells (HUVECs) and Schwann cell-laden scaffolds, such as extruded gelatin and sodium alginate composite scaffolds, have been developed to promote the recovery of injured peripheral nerves, [37–39].

Stereolithography-based 3D printing Utilizing laser beam scanning or image projection modeling, stereolithography-based 3D printing deposits cell-laden bioink in a reservoir with precise control of deposition. In general, the resolution is ~2.5–200 μm [33,40]. Nowadays, some optimized stereolithography 3D printers have been applied to construct NGCs, such as a laser-based microstereolithography (mSL) printer with a resolution of 50 μm and a projection stereolithography (PSLA) system with a resolution of 15 μm [41,42]. Particularly, digital light processing (DLP) 3D printing is developed by applying digital micromirror devices (DMDs) as a dynamic pattern generator. In DLP 3D printing an entire layer of material is simultaneously polymerized upon light exposure, which offers excellent image stability and fidelity. The theoretical resolution of a DLP printer can reach 2.5 μm [43]. The Chen group has fabricated NGCs in different complexity and size by DLP printing, such as an anatomically appropriately sized human facial NGC and special NGCs with four linear 4 mm microchannels [44]. Our group has also fabricated hydrogel NGCs rapidly by a continuous DLP 3D printing process [45]. In general, due to the special photocuring characteristics of printed biomaterials, the availability of proper bioinks for stereolithography-based 3D printing is limited.

Indirect 3D printing Comparing the above three methods, with indirect 3D printing it is much easier to fabricate a mold that provides some simple structures for NGCs. We 3D-printed molds with a 'lock and key' structure to form cryopolymerized GelMA NGCs in different shapes [46], such as multichannel and bifurcating. With the same method, Tao *et al*. also constructed a porous conduit with shape-memory property, and found that the conduits could facilitate the functional recovery of the transected model after neurorrhaphy [47]. Other researchers also utilized sacrificial molding to construct structures, such as 3D printed sacrificial frameworks of poloxamer 407 and gelatin [48,49]. Indirect 3D printing is convenient and low-cost for manufacturing. However, it is difficult to fabricate complex personalized NGCs with high resolution.

Strategies for 3D printing functional conduits

Structure design Structural cues of NGCs play an important role in physically supporting and connecting the injured nerves in peripheral nerve repair. The design of conduit structures can be divided into biomimic structures and personally customized structures, to make the reproduction of complex nerve features possible.

Biomimic structures In general, the biomimic structure of NGCs can be classified as hollow [56], multi-channel [57–59] or porous [60]. Hollow conduits with the simplest structure are the most widely used in peripheral nerve repair, some of which have been approved in clinic. However, they are not favorable for directional alignment of axons and exchange of nutrients and growth factors. It has been demonstrated that multichannel conduits are superior for axonal regeneration as they control axonal dispersion and decrease mismatch

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022

[61], especially 4-channel conduits [44,62]. However, it has also been reported that internal lumen diameter, materials and the physical properties of multi-lumen conduits can also influence axon regeneration [52]. In addition, porous NGCs could facilitate nutrient exchange which may be beneficial for nerve repair. NGCs with multi-pore is not easy to be fabricated by 3D printing. It often need to integrate with other approaches such as salt leaching and solvent casting.

Furthermore, some topographical cues of biomimic structures have also been introduced into the structural design of NGCs. It has been reported that the longitudinal groove topography of the biomaterial surface could promote the directional alignment and migration of Schwann cells as well as the neurite orientation and elongation of pheochromacytoma 12 (PC12) cells *in vitro* [63–67]. Behbehani *et al*. provided a potential *in vitro* model to explore different dimensions of grooved conduits and evaluate the performances of different cells [68]. Meanwhile, NGCs with different sizes of grooved structures on the inner surface have also been applied for nerve repair *in vivo* [69]. The smaller ones, such as nanogrooves [70] and 3 μm stripe micropatterns on the inner wall of NGCs [71], were demonstrated to effectively facilitate sciatic nerve regeneration. Most of these grooved conduits were prepared by electrospinning. For 3D printing fabrication, NGCs with microgroove structure on the inner wall were manufactured by laser-based mSL with a size resolution of 50 μm, which could support the growth, differentiation and orientation of dorsal root ganglions (DRG) *in vitro* and facilitate nerve repair across a 3 mm defect [41]. The results demonstrated that 3D printed customized grooved conduits had great potential for functional nerve repair. However, the 3D printing technologies for customized grooved conduits should be further improved.

Personally customized structures Functional NGCs with the complex anatomical structures, like branched sciatic nerve and trifacial nerve, could improve the recovery efficacy of injured nerves. In a study, researchers fabricated 'Y' shaped NGCs to match inherent tissue anatomies by microextrusion printing. Through customizing the geometries of the scaffolds and boosting function with biomimetic microgrooves and bioactive gradients, they found that the 3D printed functional NGCs could accelerate the regeneration of injured nerves by multiple nerve pathways [35]. The researchers 3D printed a Y-tube with the interposed nerve autograft, which could effectively prevent the formation of neuroma, demonstrating great potential for surgical treatment of traumatic neuromas [72]. Using advanced DLP 3D printing, Zhu *et al*. fabricated a life-size biomimetic multi-branched NGC (5.5 cm in length) featuring different branches of a complex branched human facial nerve, showing great potential for the design of personally structured NGCs [44].

Scaffold material characteristics The materials of NGCs are also critical for nerve repair, and the ideal is that they should closely mimic the native environment in order to achieve the best possible induction of nerve regeneration [73]. In general, the material for 3D printing conduits can be subcategorized into natural and synthetic. The natural polymers for conduits mainly include collagen, chitosan, gelatin, silk fibroin and hyaluronic acid, while the most commonly used synthetic ones are PCL [74], PLA [75], polyglycolic acid (PGA) and poly (lactic-co-glycolic acid) (PLGA) [76]. This section summarizes recent research into the characteristics of NGCs scaffold material, including biocompatibility, mechanical properties and conductivity.

Biocompatibility Biocompatibility is a basic requirement of NGCs. Namely, when the conduit is implanted *in vivo*, it should provide a matrix to promote cell adhesion, differentiation and growth, but not cause toxic reactions, such as hemolysis, coagulation or immune response. Generally, matrix materials usually offer bioactive properties, which can actively regulate cell behaviors and functions, including adhesion, proliferation, differentiation and migration. In particular, the cell-binding domain arginyl-glycyl-aspartic acid (RGD) peptide motif of gelatin and collagen is of great importance. Tao *et al*. fabricated NGCs with GelMA, which showed good compatibility with HUVECs and Schwann cells, and further enhanced the effect for nerve repair by incorporating drug-loaded nanoparticles [45]. Ning *et al*. used RGD chemically modified alginate with hyaluronic acid (HA) and fibrinogen as hydrogel precursors. Bioprinted Schwann cells and DRG neurons showed satisfactory cell viability and morphology [77]. In another study, substrates functionalized with I_3QGK peptide nanofibers enhanced PC12 cells attachment, proliferation and differentiation, and showed potential for nerve regeneration [78]. In fact, the degradation rate of the biomaterial should closely match the regeneration rate of nerve to avoid injury caused by the second retrieval surgery. Most degradable biomaterials can be biodegraded by hydrolase and the products should be at least not harmful for the organism; such materials include some synthetic materials and most natural materials, like PCL, PLGA and collagen. Gong *et al*. sutured rat sciatic nerves with GelMA nerve conduits which were functionalized with engelbreth-holm-swarm (EHS) hydrogel. The conduits slowly degraded and completely disappeared after 16 weeks, and exhibited favorable performance to promote the regeneration of injured sciatic nerve [55]. In a study, Singh *et al*. 3D-printed poly (glycerol sebacate methacrylate) NGCs to repair a 3 mm gap of Thy-1-YFP-H mice, and this synthetic material could be slowly degraded by lipase [54].

Mechanical properties Mechanical properties are essential characteristics for surgical operation with NGCs. Furthermore, the substrate stiffness of NGCs has been proved to have a significant influence on neuronal cells, including cell survival, spreading, adhesion, migration, neurotrophic function and average neurite length [79–82]. A study showed that rat Schwann cell precursor line (SpL201) cells presented better performance of adhesion and proliferation on stiffer substrates (in the range 1–100 MPa), while PC12 cells preferred softer ones. This might be due to mechano-transduction mechanisms involving integrins, focal

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022

adhesions and actin–myosin-associated signal pathways [79,83,84]. However, for more details, Wu *et al*. reported that 10 wt% GelMA hydrogels exhibited a Young's modulus of 34.9 KPa. This modulus was optimal for nerve regeneration of PC12 cells, including outgrowth characteristics and morphology [85]. Otherwise, with an elastic modulus of 7.45 kPa, the polyacrylamide gel substrate represented the most suitable physical support for nerve repair to regulate the biological neurotrophic behavior and function of Schwann cells [86]. The differences were caused by different substrate material compositions, cell types and specific microenvironment for cell survival. The effects of hydrogel stiffness gradient fractions on neural cell orientation and behavior have also been explored in recent research [87,88].

Conductivity In addition, electrical stimulation in particular has been demonstrated to control directional migration of neuronal and glial cells, neurite extension and differentiation of stem cells, providing insights into nerve regeneration. Recently, some conductive polymers, such as polypyrrole (PPy) and poly (acrylic acid) (PAA), have been blended with other degradable polymers (PCL) to fabricate conductive NGCs by electrohydrodynamic jet 3D printing. These conductive conduits exhibited favorable performance in neuronal cell proliferation and stem cell differentiation [74,89–91]. In addition, carbon-based conductive materials, such as black phosphorus [92], carbon nanotubes and graphene [93,94] can also be doped to matrix material. Pampaloni *et al*. demonstrated that single-layer graphene deposited on electrically insulating substrates can alter neuronal excitability by changing membrane-associated functions [94]. And Park *et al*. prepared hybrid materials by dispersing reduced graphene oxide (rGO) in GelMA hydrogel and found that rGO/GelMA with good conductivity and mechanical properties could promote the neurite outgrowth of PC12 cells. Further, the rGO/GelMA-based NGCs could improve nerve recovery in electrophysiology, gastrocnemius muscle weight and the sciatic nerve function index [95]. Apart from conductive biomaterials, piezoelectric materials which provide *in situ* artificial electrical stimulation, have also been explored for peripheral nerve regeneration, such as zinc oxide [96] and polyvinylidene fluoride [97]. However, neurotoxicity should be highlighted for all of these conductive materials, as how to minimize toxicity or explore non-toxic conductive materials is very important [98].

To construct a favorable microenvironment for maximal PNI recovery, it is necessary to consider all of the matrix materials' biocompatibility, biodegradability, conductivity and mechanical properties for NGCs. At the same time, the matrix materials must be printable to meet the need of different 3D printers, including viscosity, gelation methods and rheological properties. Currently, the characteristics of most existing materials are still not ideal. Thus, a combination of two or three kinds of materials which have special functions that can synergistically functionalize the NGCs may be an effective strategy.

Functional biological cues Another potential strategy for efficient nerve recovery is to functionalize the customized NGCs with biological cues including cells, exogenous bioactive factors and drugs (Table 2). These functional biological cues could improve neural signals around or through the injured region in some cases and provide a more permissive environment for nerve regeneration.

Live cells Cell-based therapy has great application potential in PNI repair. Different types of cells that are beneficial to injured nerve regeneration, including glial cells and stem cells, have been integrated into NCGs in recent decades [99]. Schwann cells, a native glial population that can form myelin sheath and secrete neurotrophic factors, are neuroprotective and have been widely applied in PNI repair [100]. In a study, the nanocomposite-based NGCs loaded with the rat Schwann cell line could considerably improve axonal myelination and nerve regeneration, as shown by analysis of regenerated nerves [101]. In another study, native glial olfactory ensheathing cells (OECs) were seeded in perfluorotributylamine-loaded collagen–chitosan NGCs. The results showed this strategy could increase oxygen supply and promote axon regeneration [102]. Recently, Wu *et al*. 3D bioprinted a composite scaffold with hybrid bioinks of gelatin, sodium alginate and Schwann cells. They found that the 3D scaffold significantly enhanced cell adhesion and upregulated the level of related neurotrophic factors compared to 2D culture, which showed promising application of 3D bioprinted NGCs with cells [37].

In addition, different kinds of stem cells such as mesenchymal stem cells (MSCs), adipose-derived stem cells (ADSCs), neural stem cells (NSCs) and induced pluripotent stem cells (iPSCs) have been encapsulated into NGCs for PNI repair [103]. Zhang *et al*. combined MSCs with erythropoietin-loaded chitosan nerve conduits and found this strategy could accelerate nerve repair and morphological recovery *in vivo* [104]. Hu *et al*. seeded ADSCs on the cryoGelMA conduit and the results demonstrated that the gene expression of major neurotrophic factors such as brain-derived neurotrophic facters (BDNF) was significantly up-regulated in the cells. Besides, other stem cells, like bone marrow-derived stem cells (BMSC), skin derived stem cells (SDSC) and amniotic fluid mesenchymal stem cells (AFMSC), have also exhibited great potential in repair of PNI [105–108]. Qian *et al*. showed that the differentiation of BMSC into Schwann-like cells was facilitated by PCL NGCs that were functionalized with Au nanoparticles and polydopamine (PDA) [60].

Growth factors Growth factors as another biological cue are important for functional nerve repair. Various kinds of growth factors have been incorporated into NGCs to improve the recovery efficacy, including nerve growth factors (NGF), glial cell line-derived neurotrophic factor (GDNF), ciliary neurotrophic factors (CNTF), BDNF, fibroblast growth factors (FGF) etc. [109–111]. These factors in conduits can be released to regulate the function of transplanted cells and

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022

Table 2. Biological cues in conduits for nerve repair

Additive	Type	Material	Gap size	Treatment time	Application	Reference
Schwann cell	Cell	Silk–gold nanocomposite	10 mm	18 months	Enhancing myelination of the regenerated nerves	[101]
Neural crest stem cell	Cell	Polycaprolactone nanofiber	15 mm	6/12 weeks	Promoting regeneration across large nerve gaps	[105]
MSC	Cell	EPO-loaded Chitosan	5 mm	4/8 weeks	Accelerating nerve healing process	[104]
ASC	Cell	Cryopolymerized gelatin methacryloyl	10 mm	4/8/16 weeks	Providing a conducive environment for nerve recovery	[46]
OEC	Cell	Collagen–chitosan	15 mm	12 weeks	Promoting axonal regeneration and functional recovery	[102]
CNTF/bFGF	Factor	Collagen	35 mm in minipig	6 months	Repairing 35 mm gap in minipigs	[113]
NGF	Factor	PCLA/silk fibroin	15 mm	12 weeks	Significant improvements in promoting and guiding neurite outgrowth	[114]
GDNF	Factor	PCL	5 cm	1 year	Increasing nerve conduction velocity and average area occupied by individual Schwann cells	[115]
Platelet	Cytoplasm	GelMA/PEGDA	10 mm	12 weeks	Promoting hydrogel conduits in peripheral nerve repair	[56]
Melatonin	Drug	PCL	15 mm	12 weeks	Improving recovery efficiency by inhibiting oxidative stress and inflammation	[118]
XMU-MP-1	Drug	GelMA	10 mm	3 months	Promoting the proliferation, migration and neurotrophic factors secretion of Schwann cells	[45]
PSA/HNK-1	Drug	Polyethylene	5 mm	8/15 weeks	Increasing the ratio of myelinated axons/improvements in myelination	[121]
RGFP966	Drug	GelMA	10 mm	3 months	Efficiently bridging a 10-mm gap of rat sciatic nerve by promoting remyelination of axons	[119]

MSC mesenchymal stem cell, *EPO* epoetin, *ADSC* adipose-derived stem cell, *OEC* olfactory ensheathing cell, *CNTF/bFGF* ciliary neurotrophic factor and basic fibroblast growth factor, *NGF* nerve growth factors, *PCLA* poly(ε-caprolactone-co-lactide), *GDNF* glial cell line-derived neurotrophic factor, *GelMA/PEGDA* gelatin methacrylate and polyethylene glycol diacrylate, *PCL* poly(ε-caprolactone), *PSA/HNK-1* polysialic acid and human natural killer cell-1

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022

host cells through different signaling pathways [112]. Specifically, they can improve the survival, proliferation and migration of transplanted cells, promote the infiltration of host cells, enhance the neuronal differentiation of stem cells and improve the neural regenerative capacities of Schwann cells [99]. This may provide a more beneficial biological microenvironment for nerve repair. Cui *et al*. reported that the collagen NGCs containing CNTF and basic fibroblast growth factor (bFGF) could promote PNI repair across a 35 mm distance in minipig models [113]. The concentration gradients or distribution of the growth factors could affect the repair efficacy. In a study, NGCs functionalized with concentration gradients of NGF exhibited superior outcomes of 15 mm-long-gap nerve regeneration than those of NGCs with uniform NGF [114]. In addition, long-term sustained-release of the growth factors from conduits may construct a temporal-beneficial microenvironment for the recovery of injured nerves. Fadia *et al*. fabricated PCL NGCs with GDNF microspheres that provided sustained release of GDNF in a 5 cm nerve defect of rhesus macaques for >50 days [115]. Interestingly, in a recent study, Tao *et al*. 3D-printed functional NGCs by incorporating bioinks with live platelets isolated from whole blood. This kind of conduit could slow down the activation of the live platelets and sustainedly release of multiple growth factors, which significantly improved nerve recovery *in vivo* [56].

Drugs Currently, several pharmaceuticals have been approved by the FDA for PNI therapy through local injection or systemic administration, but effective results have not been achieved. Alternatively, drug-loaded NGCs can provide sustained release of a drug locally and reduce systemic side-effects; they typically stimulate nerve regeneration and myelination by acting on targets, activating signaling pathways, and promoting the growth and proliferation of the cells in volved in the regeneration process [116]. Melatonin can reduce oxidative stress and inhibit inflammation after nerve injury thus promoting the recovery of peripheral nerves [117]. 3D printed melatonin/PCL NGCs exhibited better biocompatibility than PCL NGCs by accelerating the proliferation of Schwann cells and upregulating the gene expression of neural-related proteins in the cells [117,118]. Recently, some small molecules have been reported to be functional in accelerating nerve regeneration and recovery. Tao *et al*. functionalized 3D-printed NGCs with XMU-MP-1, and found that this small molecule could promote the functional recovery of injured nerves through inhibiting the Hippo pathway [45]. Xu *et al*. 3D-printed NGCs incorporating RGFP966-loaded nanoparticles, and demonstrated that RGFP966 could efficiently repair the injured nerve by activating the PI3K-AKT-ERK signaling pathway to enhance the remyelination of Schwann cells [119]. It has also been reported that incorporating drugs within the luminal filler of the conduits can also promote nerve regeneration after injury [55,120,121].

3D printing technology is a convenient method that can be used to fabricate different morphological NGCs that could influence neural cell proliferation and migration [122]. Additionally, the microstructures of NGCs can also affect the controlled and sustained release of growth factors and drugs to offer specific cues to the regenerating tissues [123]. Integrating cells, growth factors and drugs into biomaterials to prepare functional NGCs may be a potential strategy to build a more ideal microenvironment for functional recovery of injured nerves. However, the problems of cell viability, stability of growth factors and controlled release of additives that greatly affect the efficiency of nerve regeneration should be considered.

Conclusions

The ideal functional NGCs require physico-chemical cues and biological cues to synergistically construct a biomimetic microenvironment and maintain physiological balance for nerve repair. The emerging 3D printing technology with design flexibility, personalized customization and controllable manufacturing precision is an advanced method to achieve the functionalization of NGCs. Currently, remarkable advances have been made in the fabrication and functionalization of 3D printed NGCs and their corresponding applications in peripheral nerve repair and regeneration. However, there are still some challenges for the future. Firstly, to precisely fabricate customized functional NGCs, the printing system should be optimized, especially with regard to precision and speed. Secondly, the bioinks for NGCs are limited. The characteristics of printable materials for conduits, such as biodegradation, biocompatibility and mechanical properties, need to be further improved. Using a combination of different biomaterials to fabricate suitable NGCs could be a choice for multifunctional conduits. Thirdly, aiming for clinical translation of neural tissue engineering, fabricating human-scale constructs for long-gap injuries and big animal models for PNI still remain a great challenge. What is more, exploring and clarifying the mechanisms of the repair of injured nerves is of great significance and could offer direction on how to design new strategies for NGCs. Generally, the gradual advancement in biomaterials and 3D printing techniques provide promise for the fabrication of human-scale customized constructs for long-gap PNI with ideal therapeutic effect.

Abbreviations

ADSC: Adipose-derived stem cells; BDNF: Brain-derived neurotrophic factors; BMSC: Bone marrow-derived stem cells; CNTF: Ciliary neurotrophic factors; DLP: Digital light processing; GDNF: Glial cell line-derived neurotrophic factor; MSC: Mesenchymal stem cells; mSL: Microstereolithography; NGC: Nerve guide conduits; NGF: Nerve growth factors; PLA: Polylactic acid; PCL: Poly (ε-caprolactone); PLGA: Poly (lactic-co-glycolic acid); PNI: Peripheral nerve injury; rGO: Reduced graphene oxide

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022

Funding

This work was funded by 1.3.5 project for disciplines of excellence, West China Hospital, Sichuan University (ZYJC18017, ZYYC08007), China Postdoctoral Science Foundation (2020M673229) and Post-Doctor Research Project, West China Hospital, Sichuan University (2020HXBH064, 2020HXBH025).

Conflict of interest

The authors declare that they have no competing interests.

References

1. Rigoni M, Negro S. Signals orchestrating peripheral nerve repair. *Cells*. 2020;9:1768.
2. Carvalho CR, Silva-Correia J, Oliveira JM, Reis RL. Nanotechnology in peripheral nerve repair and reconstruction. *Adv Drug Deliv Rev*. 2019;148:308–43.
3. Mukhatyar V, Karumbaiah L, Yeh J, Bellamkonda R. *Tissue Engineering Strategies Designed to Realize the Endogenous Regenerative Potential of Peripheral Nerves*. Advanced Materials. 2009;21:4670–79.
4. Chandran V, Coppola G, Nawabi H, Omura T, Versano R, Huebner EA, *et al.* A systems-level analysis of the peripheral nerve intrinsic axonal growth program. *Neuron*. 2016;89:956–70.
5. Griffin JW, Hogan MV, Chhabra AB, Deal DN. Peripheral nerve repair and reconstruction. *J Bone Joint Surg Am*. 2013;95:2144–51.
6. Raza C, Riaz HA, Anjum R, Shakeel NA. Repair strategies for injured peripheral nerve: review. *Life Sci*. 2020;243:117308.
7. Deumens R, Bozkurt A, Meek MF, Marcus MA, Joosten EA, Weis J, *et al.* Repairing injured peripheral nerves: bridging the gap. *Prog Neurobiol*. 2010;92:245–76.
8. Yin J, Zhang DM, Xiang Y, Wei P, Yang ZY, Wang ZH, *et al.* The influence of cross-sectional morphology on the compressive resistance of polymeric nerve conduits. *Polymer*. 2018;148:93–100.
9. Kehoe S, Zhang XF, Boyd D. FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy. *Injury*. 2012;43:553–72.
10. Kornfeld T, Vogt PM, Radtke C. Nerve grafting for peripheral nerve injuries with extended defect sizes. *Wien Med Wochenschr*. 2019;169:240–51.
11. Bellamkonda RV. Peripheral nerve regeneration: An opinion on channels, scaffolds and anisotropy. *Biomaterials*. 2006;27:3515–8.
12. Zhang X, Qu W, Li D, Shi K, Li R, Han Y, *et al.* Functional polymer-based nerve guide conduits to promote peripheral nerve regeneration. *Adv Mater Interfaces*. 2020;7:2000225.
13. Manoukian OS, Baker JT, Rudraiah S, Arul MR, Vella AT, Domb AJ, *et al.* Functional polymeric nerve guidance conduits and drug delivery strategies for peripheral nerve repair and regeneration. *J Control Release*. 2020;317:78–95.
14. Ma X, Liu J, Zhu W, Tang M, Lawrence N, Yu C, *et al.* 3D bioprinting of functional tissue models for personalized drug screening and in vitro disease modeling. *Adv Drug Deliv Rev*. 2018;132:235–51.
15. Murphy SV, Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol*. 2014;32:773–85.
16. Kang HW, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotechnol*. 2016;34:312–9.
17. Suo HR, Li L, Zhang CX, Yin J, Xu KD, Liu JY, *et al.* Glucosamine-grafted methacrylated gelatin hydrogels as potential biomaterials for cartilage repair. *Journal of Biomedical Materials Research Part B-Applied Biomaterials*. 2020;108:990–9.
18. Grigoryan B, Paulsen SJ, Corbett DC, Sazer DW, Fortin CL, Zaita AJ, *et al.* BIOMEDICINE multivascular networks and functional intravascular topologies within biocompatible hydrogels. *Science*. 2019;364:458–64.
19. Noor N, Shapira A, Edri R, Gal I, Wertheim L, Dvir T. 3D printing of personalized thick and Perfusable cardiac patches and hearts. *Adv Sci*. 2019;6:1900344.
20. Maiti B, Diaz Diaz D. 3D printed polymeric hydrogels for nerve regeneration. *Polymers*. 2018;10:1041.
21. Ford S, Despeisse M. Additive manufacturing and sustainability: an exploratory study of the advantages and challenges. *J Clean Prod*. 2016;137:1573–87.
22. Wang X, Jiang M, Zhou ZW, Gou JH, Hui D. 3D printing of polymer matrix composites: a review and prospective. *Composites Part B-Engineering*. 2017;110:442–58.
23. Sarker M, Naghieh S, McInnes AD, Schreyer DJ, Chen X. Strategic design and fabrication of nerve guidance conduits for peripheral nerve regeneration. *Biotechnol J*. 2018;13:1700635.
24. Xue JJ, Wu T, Dai YQ, Xia YN. Electrospinning and electrospun nanofibers: methods, materials, and applications. *Chem Rev*. 2019;119:5298–415.
25. Ling L, Yang Y. Exploration on creative product customization design based on 3D printing technology research. *Appl Mech Mater*. 2015;709:509–12.
26. Ghosh U, Ning S, Wang Y, Kong YL. Addressing unmet clinical needs with 3D printing technologies. *Adv Healthc Mater*. 2018;7:1800417.
27. Rengier F, Mehndiratta A, von Tengg-Kobligk H, Zechmann CM, Unterhinninghofen R, Kauczor HU, *et al.* 3D printing based on imaging data: review of medical applications. *Int J Comput Assist Radiol Surg*. 2010;5:335–41.
28. Felix HM, Rosenbush KA, Lannen AM, Pooley RA, Siegel JL, Brown BL, *et al.* Creation of an optic nerve sheath diameter ultrasound model for NeuroICU education. *Mil Med Res*. 2020;7:43.
29. Gu Q, Zhu H, Li J, Li X, Hao J, Wallace GG, *et al.* Three-dimensional bioprinting speeds up smart regenerative medicine. *Natl Sci Rev*. 2016;3:331–44.
30. Yu X, Zhang T, Li Y. 3D printing and bioprinting nerve conduits for neural tissue engineering. *Polymers*. 2020;12:1637.
31. Radulescu D, Dhar S, Young CM, Taylor DW, Trost H-J, Hayes DJ, *et al.* Tissue engineering scaffolds for nerve regeneration manufactured by ink-jet technology. *Mater Sci Eng C*. 2007;27:534–9.
32. Tse C, Whiteley R, Yu T, Stringer J, MacNeil S, Haycock JW, *et al.* Inkjet printing Schwann cells and neuronal analogue NG108-15 cells. *Biofabrication*. 2016;8:015017.
33. Cui H, Nowicki M, Fisher JP, Zhang LG. 3D bioprinting for organ regeneration. *Adv Healthc Mater*. 2017;6:1601118.

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022

34. Wang R, Wang Y, Yao B, Hu T, Li Z, Liu Y, *et al.* Redirecting differentiation of mammary progenitor cells by 3D bioprinted sweat gland microenvironment. *Burns Trauma*. 2019;7:29.
35. Johnson BN, Lancaster KZ, Zhen G, He J, Gupta MK, Kong YL, *et al.* 3D printed anatomical nerve regeneration pathways. *Adv Funct Mater*. 2015;25:6205–17.
36. Jakus AE, Secor EB, Rutz AL, Jordan SW, Hersam MC, Shah RN. Three-dimensional printing of high-content graphene scaffolds for electronic and biomedical applications. *ACS Nano*. 2015;9:4636–48.
37. Wu Z, Li Q, Xie S, Shan X, Cai Z. In vitro and in vivo biocompatibility evaluation of a 3D bioprinted gelatin-sodium alginate/rat Schwann-cell scaffold. *Korean J Couns Psychother*. 2020;109:110530–0.
38. Li X, Wang X, Wang X, Chen H, Zhang X, Zhou L, *et al.* 3D bioprinted rat schwann cell-laden structures with shape flexibility and enhanced nerve growth factor expression. *3 Biotech*. 2018;8:342.
39. Soltan N, Ning L, Mohabatpour F, Papagerakis P, Printability CX. Cell viability in bioprinting alginate Dialdehyde-Gelatin scaffolds. *ACS Biomater Sci Eng*. 2019;5:2976–87.
40. Ligon SC, Liska R, Stampfl J, Gurr M, Muelhaupt R. Polymers for 3D printing and customized additive manufacturing. *Chem Rev*. 2017;117:10212–90.
41. Pateman CJ, Harding AJ, Glen A, Taylor CS, Christmas CR, Robinson PP, *et al.* Nerve guides manufactured from photocurable polymers to aid peripheral nerve repair. *Biomaterials*. 2015;49:77–89.
42. Singh A, Asikainen S, Teotia AK, Shiekh PA, Huotilainen E, Qayoom I, *et al.* Biomimetic Photocurable three-dimensional printed nerve guidance channels with aligned Cryomatrix lumen for peripheral nerve regeneration. *ACS Appl Mater Interfaces*. 2018;10:43327–42.
43. Lee SJ, Esworthy T, Stake S, Miao S, Zuo YY, Harris BT, *et al.* Advances in 3D bioprinting for neural tissue engineering. *Advanced Biosystems*. 2018;2:1700213.
44. Zhu W, Tringale KR, Woller SA, You S, Johnson S, Shen H, *et al.* Rapid continuous 3D printing of customizable peripheral nerve guidance conduits. *Mater Today*. 2018;21:951–9.
45. Tao J, Zhang J, Du T, Xu X, Deng X, Chen S, *et al.* Rapid 3D printing of functional nanoparticle-enhanced conduits for effective nerve repair. *Acta Biomater*. 2019;90:49–59.
46. Hu Y, Wu Y, Gou Z, Tao J, Zhang J, Liu Q, *et al.* 3D-engineering of Cellularized conduits for peripheral nerve regeneration. *Sci Rep*. 2016;6:32184.
47. Tao J, Hu Y, Wang S, Zhang J, Liu X, Gou Z, *et al.* A 3D-engineered porous conduit for peripheral nerve repair. *Sci Rep*. 2017;7:46038.
48. Mueller M, Becher J, Schnabelrauch M, Zenobi-Wong M. Printing thermoresponsive reverse molds for the creation of patterned two-component hydrogels for 3D cell culture. *J Vis Exp*. 2013;77:e50632.
49. Naghieh S, Sarker MD, Abelseth E, Chen X. Indirect 3D bioprinting and characterization of alginate scaffolds for potential nerve tissue engineering applications. *J Mech Behav Biomed Mater*. 2019;93:183–93.
50. Xu T, Gregory CA, Molnar P, Cui X, Jalota S, Bhaduri SB, *et al.* Viability and electrophysiology of neural cell structures generated by the inkjet printing method. *Biomaterials*. 2006;27:3580–8.
51. Zhang AP, Qu X, Soman P, Hribar KC, Lee JW, Chen S, *et al.* Rapid fabrication of complex 3D extracellular microenvironments by dynamic optical projection stereolithography. *Adv Mater*. 2012;24:4266–70.
52. Evangelista MS, Perez M, Salibian AA, Hassan JM, Darcy S, Paydar KZ, *et al.* Single-lumen and multi-lumen poly(ethylene glycol) nerve conduits fabricated by Stereolithography for peripheral nerve regeneration in vivo. *J Reconstr Microsurg*. 2015;31:327–35.
53. Lee S-J, Nowicki M, Harris B, Zhang LG. Fabrication of a highly aligned neural scaffold via a table top Stereolithography 3D printing and electrospinning. *Tissue Eng Part A*. 2017;23:491–502.
54. Singh D, Harding AJ, Albadawi E, Boissonade FM, Haycock JW, Claeyssens F. Additive manufactured biodegradable poly (glycerol sebacate methacrylate) nerve guidance conduits. *Acta Biomater*. 2018;78:48–63.
55. Gong H, Fei H, Xu Q, Gou M, Chen HH. 3D-engineered GelMA conduit filled with ECM promotes regeneration of peripheral nerve. *J Biomed Mater Res A*. 2020;108:805–13.
56. Tao J, Liu H, Wu W, Zhang J, Liu S, Zhang J, *et al.* 3D-printed nerve conduits with live platelets for effective peripheral nerve repair. *Adv Funct Mater*. 2020;30:2004272.
57. Wang J, Xiong H, Zhu T, Liu Y, Pan H, Fan C, *et al.* Bioinspired multichannel nerve guidance conduit based on shape memory nanofibers for potential application in peripheral nerve repair. *ACS Nano*. 2020;14:12579–95.
58. Sun X, Bai Y, Zhai H, Liu S, Zhang C, Xu Y, *et al.* Devising micro/nano-architectures in multi-channel nerve conduits towards a pro-regenerative matrix for the repair of spinal cord injury. *Acta Biomater*. 2019;86:194–206.
59. Diez-Ahedo R, Mendibil X, Carmen Marquez-Posadas M, Quintana I, Gonzalez F, Javier Rodriguez F, *et al.* UV-casting on Methacrylated PCL for the production of a peripheral nerve implant containing an Array of porous aligned microchannels. *Polymers*. 2020;12:971.
60. Qian Y, Song J, Zheng W, Zhao X, Ouyang Y, Yuan W, *et al.* 3D manufacture of gold nanocomposite channels facilitates neural differentiation and regeneration. *Adv Funct Mater*. 2018;28:1707077.
61. Yao L, de Ruiter GC, Wang H, Knight AM, Spinner RJ, Yaszemski MJ, *et al.* Controlling dispersion of axonal regeneration using a multichannel collagen nerve conduit. *Biomaterials*. 2010;31:5789–97.
62. Arcaute K, Mann BK, Wicker RB. Fabrication of off-the-shelf multilumen poly(ethylene glycol) nerve guidance conduits using Stereolithography. *Tissue Engineering Part C-Methods*. 2011;17:27–38.
63. He J, Sun C, Gu Z, Yang Y, Gu M, Xue C, *et al.* Morphology, migration, and transcriptome analysis of Schwann cell culture on butterfly wings with different surface architectures. *ACS Nano*. 2018;12:9660–8.
64. Jiang T, Zhao J, Yu S, Mao Z, Gao C, Zhu Y, *et al.* Untangling the response of bone tumor cells and bone forming cells to matrix stiffness and adhesion ligand density by means of hydrogels. *Biomaterials*. 2019;188:130–43.
65. Zhang D, Xu S, Wu S, Gao C. Micropatterned poly (d,l-lactide-co-caprolactone) films entrapped with gelatin for promoting the alignment and directional migration of Schwann cells. *J Mater Chem B*. 2018;6:1226–37.

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022

66. Zhang D, Wu S, Feng J, Duan Y, Xing D, Gao C. Micropatterned biodegradable polyesters clicked with CQAASIKVAV promote cell alignment, directional migration, and neurite outgrowth. *Acta Biomater*. 2018;74:143–55.
67. Zhang DM, Suo HR, Qian J, Yin J, Fu JZ, Huang Y. Physical understanding of axonal growth patterns on grooved substrates: groove ridge crossing versus longitudinal alignment. *Bio-Design and Manufacturing*. 2020;3:348–60.
68. Behbehani M, Glen A, Taylor CS, Schuhmacher A, Claeyssens F, Haycock JW. Pre-clinical evaluation of advanced nerve guide conduits using a novel 3D in vitro testing model. *International Journal of Bioprinting*. 2018;4:123.
69. Wang ZH, Wu YB, Xiang Y, Kruth MB, Wei P, Dai GL, *et al.* Efficacy of large groove texture on rat sciatic nerve RegenerationIn VivoUsing Polyacrylonitrile nerve conduits. *Ann Biomed Eng*. 2020;49:394–406.
70. Huang C, Ouyang Y, Niu H, He N, Ke Q, Jin X, *et al.* Nerve guidance conduits from aligned nanofibers: improvement of nerve regeneration through longitudinal Nanogrooves on a Fiber surface. *ACS Appl Mater Interfaces*. 2015;7:7189–96.
71. Zhang D, Yao Y, Duan Y, Yu X, Shi H, Nakkala JR, *et al.* Surface-anchored graphene oxide Nanosheets on cell-scale micropatterned poly (d,l-lactide-co-caprolactone) conduits promote peripheral nerve regeneration. *ACS Appl Mater Interfaces*. 2020;12:7915–30.
72. Bolleboom A, de Ruiter GCW, Coert JH, Tuk B, Holstege JC, van Neck JW. Novel experimental surgical strategy to prevent traumatic neuroma formation by combining a 3D-printed Y-tube with an autograft. *J Neurosurg*. 2018;130:184–96. doi: 10.3171/2017.8.JNS17276.
73. Jiang L-B, Su D-H, Ding S-L, Zhang Q-C, Li Z-F, Chen F-C, *et al.* Salt-assisted toughening of protein hydrogel with controlled degradation for bone regeneration. *Adv Funct Mater*. 2019;29:1901314.
74. Vijayavenkataraman S, Kannan S, Cao T, Fuh JYH, Sriram G, Lu WF. 3D-printed PCL/PPy conductive scaffolds as three-dimensional porous nerve guide conduits (NGCs) for peripheral nerve injury repair. *Front Bioeng Biotechnol*. 2019;7:266.
75. Sun B, Zhou Z, Li D, Wu T, Zheng H, Liu J, *et al.* Polypyrrole-coated poly (l-lactic acid-co-epsilon-caprolactone)/silk fibroin nanofibrous nerve guidance conduit induced nerve regeneration in rat. *Materials Science & Engineering C-Materials for Biological Applications*. 2019;94:190–9.
76. Vijayavenkataraman S. Nerve guide conduits for peripheral nerve injury repair: a review on design, materials and fabrication methods. *Acta Biomater*. 2020;106:54–69.
77. Ning L, Zhu N, Mohabatpour F, Sarker MD, Schreyer DJ, Chen X. Bioprinting Schwann cell-laden scaffolds from low-viscosity hydrogel compositions. *J Mater Chem B*. 2019;7:4538–51.
78. Sun W, Zhang Y, Gregory DA, Jimenez-Franco A, Tomeh MA, Lv S, *et al.* Patterning the neuronal cells via inkjet printing of self-assembled peptides on silk scaffolds. *Progress in Natural Science-Materials International*. 2020;30:686–96.
79. Geiger B, Spatz JP, Bershadsky AD. Environmental sensing through focal adhesions. *Nat Rev Mol Cell Biol*. 2009;10:21–33.
80. Lv HW, Wang HP, Zhang ZJ, Yang W, Liu WB, Li YL, *et al.* Biomaterial stiffness determines stem cell fate. *Life Sci*. 2017;178:42–8.
81. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, *et al.* Role of YAP/TAZ in mechanotransduction. *Nature*. 2011;474:179–U212.
82. Chaudhuri O, Gu L, Klumpers D, Darnell M, Bencherif SA, Weaver JC, *et al.* Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nat Mater*. 2016;15:326–34.
83. Cai L, Zhang L, Dong JY, Wang SF. Photocured biodegradable polymer substrates of varying stiffness and microgroove dimensions for promoting nerve cell guidance and differentiation. *Langmuir*. 2012;28:12557–68.
84. Wang SF, Kempen DHR, de Ruiter GCW, Cai L, Spinner RJ, Windebank AJ, *et al.* Molecularly engineered biodegradable polymer networks with a wide range of stiffness for bone and peripheral nerve regeneration. *Adv Funct Mater*. 2015;25:2715–24.
85. Wu YB, Xiang Y, Fang JH, Li XK, Lin ZW, Dai GL, *et al.* The influence of the stiffness of GelMA substrate on the outgrowth of PC12 cells. *Biosci Rep*. 2019;39:BSR20181748.
86. Gu Y, Ji Y, Zhao Y, Liu Y, Ding F, Gu X, *et al.* The influence of substrate stiffness on the behavior and functions of Schwann cells in culture. *Biomaterials*. 2012;33:6672–81.
87. Kayal C, Moeendarbary E, Shipley RJ, Phillips JB. Mechanical response of neural cells to physiologically relevant stiffness gradients. *Adv Healthc Mater*. 2020;9:e1901036.
88. Lavrentieva A, Fleischhammer T, Enders A, Pirmahboub H, Bahnemann J, Pepelanova I. Fabrication of stiffness gradients of GelMA hydrogels using a 3D printed micromixer. *Macromol Biosci*. 2020;20:2000107.
89. Vijayavenkataraman S, Zhang S, Thaharah S, Sriram G, Lu WF, Fuh JYH. Electrohydrodynamic jet 3D printed nerve guide conduits (NGCs) for peripheral nerve injury repair. *Polymers*. 2018;10:753.
90. Vijayavenkataraman S, Thaharah S, Zhang S, Lu WF, Fuh JYH. 3D-printed PCL/rGO conductive scaffolds for peripheral nerve injury repair. *Artif Organs*. 2019;43:515–23.
91. Vijayavenkataraman S, Thaharah S, Zhang S, Lu WF, Fuh JYH. Electrohydrodynamic jet 3D-printed PCL/PAA conductive scaffolds with tunable biodegradability as nerve guide conduits (NGCs) for peripheral nerve injury repair. *Materials & Design*. 2019;162:171–84.
92. Qian Y, Yuan W-E, Cheng Y, Yang Y, Qu X, Fan C. Concentrically integrative bioassembly of a three-dimensional black phosphorus Nanoscaffold for restoring neurogenesis. *Angiogenesis, and Immune Homeostasis Nano Letters*. 2019;19:8990–9001.
93. Qian Y, Zhao X, Han Q, Chen W, Li H, Yuan W. An integrated multi-layer 3D-fabrication of PDA/RGD coated graphene loaded PCL nanoscaffold for peripheral nerve restoration. *Nat Commun*. 2018;9:323.
94. Pampaloni NP, Lottner M, Giugliano M, Matruglio A, D'Amico F, Prato M, *et al.* Single-layer graphene modulates neuronal communication and augments membrane ion currents. *Nat Nanotechnol*. 2018;13:755–64.
95. Park J, Jeon J, Kim B, Lee MS, Park S, Lim J, *et al.* Electrically conductive hydrogel nerve guidance conduits for peripheral nerve regeneration. *Adv Funct Mater*. 2020;30.
96. Qian Y, Cheng Y, Song J, Xu Y, Yuan W-E, Fan C, *et al.* Mechano-informed biomimetic polymer scaffolds by incorporating self-powered zinc oxide Nanogenerators

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022

enhance motor recovery and neural function. *Small*. 2020;16: e2000796.
97. Cheng Y, Xu Y, Qian Y, Chen X, Ouyang Y, Yuan W-E. 3D structured self-powered PVDF/PCL scaffolds for peripheral nerve regeneration. *Nano Energy*. 2020;69:104411.
98. Fei J, Wen X, Lin X, Saijilafu WW, Ren O, *et al*. Biocompatibility and neurotoxicity of magnesium alloys potentially used for neural repairs. *Materials Science & Engineering C-Materials for Biological Applications*. 2017;78:1155–63.
99. Wang ZZ, Sakiyama-Elbert SE. Matrices, scaffolds & carriers for cell delivery in nerve regeneration. *Exp Neurol*. 2019;319:112837.
100. Cerqueira SR, Lee YS, Cornelison RC, Mertz MW, Wachs RA, Schmidt CE, *et al*. Decellularized peripheral nerve supports Schwann cell transplants and axon growth following spinal cord injury. *Biomaterials*. 2018;177:176–85.
101. Das S, Sharma M, Saharia D, Sarma KK, Sarma MG, Borthakur BB, *et al*. In vivo studies of silk based gold nano-composite conduits for functional peripheral nerve regeneration. *Biomaterials*. 2015;62:66–75.
102. Zhu S, Ge J, Wang Y, Qi F, Ma T, Wang M, *et al*. A synthetic oxygen carrier-olfactory ensheathing cell composition system for the promotion of sciatic nerve regeneration. *Biomaterials*. 2014;35:1450–61.
103. Yi S, Zhang Y, Gu X, Huang L, Zhang K, Qian T, *et al*. Application of stem cells in peripheral nerve regeneration. *Burns Trauma*. 2020;8:tkaa002.
104. Zhang W, Zhang L, Liu J, Zhang L, Zhang J, Tang P. Repairing sciatic nerve injury with an EPO-loaded nerve conduit and sandwiched-in strategy of transplanting mesenchymal stem cells. *Biomaterials*. 2017;142:90–100.
105. Du J, Zhen G, Chen H, Zhang S, Qing L, Yang X, *et al*. Optimal electrical stimulation boosts stem cell therapy in nerve regeneration. *Biomaterials*. 2018;181:347–59.
106. Hu N, Wu H, Xue C, Gong Y, Wu J, Xiao Z, *et al*. Long-term outcome of the repair of 50 mm long median nerve defects in rhesus monkeys with marrow mesenchymal stem cells-containing, chitosan-based tissue engineered nerve grafts. *Biomaterials*. 2013;34:100–11.
107. Marchesi C, Pluderi M, Colleoni F, Belicchi M, Meregalli M, Farini A, *et al*. Skin-derived stem cells transplanted into resorbable guides provide functional nerve regeneration after sciatic nerve resection. *Glia*. 2007;55: 425–38.
108. Pan HC, Cheng FC, Chen CJ, Lai SZ, Lee CW, Yang DY, *et al*. Post-injury regeneration in rat sciatic nerve facilitated by neurotrophic factors secreted by amniotic fluid mesenchymal stem cells. *J Clin Neurosci*. 2007;14:1089–98.
109. Hu Z, An K, Wang J, Xu X, Chen Z, Hu J, *et al*. Preparation and drug release behavior of tussah silk fibroin composite membrane. *Fibers and Polymers*. 2020;21: 252–61.
110. Chen R, Zhou L, Yang H, Zheng H, Zhou Y, Hu Z, *et al*. Degradation behavior and immunological detection of silk fibroin exposure to enzymes. *Anal Sci*. 2019;35:1243–9.
111. Kokai LE, Ghaznavi AM, Marra KG. Incorporation of double-walled microspheres into polymer nerve guides for the sustained delivery of glial cell line-derived neurotrophic factor. *Biomaterials*. 2010;31:2313–22.
112. Nagappan PG, Chen H, Wang D-Y. Neuroregeneration and plasticity: a review of the physiological mechanisms for achieving functional recovery postinjury. *Mil Med Res*. 2020;7:30.
113. Cui Y, Lu C, Meng D, Xiao Z, Hou X, Ding W, *et al*. Collagen scaffolds modified with CNTF and bFGF promote facial nerve regeneration in minipigs. *Biomaterials*. 2014;35: 7819–27.
114. Tang S, Zhu J, Xu Y, Xiang AP, Jiang MH, Quan D. The effects of gradients of nerve growth factor immobilized PCLA scaffolds on neurite outgrowth in vitro and peripheral nerve regeneration in rats. *Biomaterials*. 2013;34:7086–96.
115. Fadia NB, Bliley JM, DiBernardo GA, Crammond DJ, Schilling BK, Sivak WN, *et al*. Long-gap peripheral nerve repair through sustained release of a neurotrophic factor in nonhuman primates. *Sci Transl Med*. 2020;12:eabc4054.
116. Bota O, Fodor L. The influence of drugs on peripheral nerve regeneration. *Drug Metab Rev*. 2019;51:266–92.
117. Chang HM, Liu CH, Hsu WM, Chen LY, Wang HP, Wu TH, *et al*. Proliferative effects of melatonin on Schwann cells: implication for nerve regeneration following peripheral nerve injury. *J Pineal Res*. 2014;56:322–32.
118. Qian Y, Han Q, Zhao X, Song J, Cheng Y, Fang Z, *et al*. 3D melatonin nerve scaffold reduces oxidative stress and inflammation and increases autophagy in peripheral nerve regeneration. *J Pineal Res*. 2018;65:e12516.
119. Xu X, Tao J, Wang S, Yang L, Zhang J, Zhang J, *et al*. 3D printing of nerve conduits with nanoparticle-encapsulated RGFP966. *Appl Mater Today*. 2019;16:247–56.
120. Pace LA, Plate JF, Smith TL, Van Dyke ME. The effect of human hair keratin hydrogel on early cellular response to sciatic nerve injury in a rat model. *Biomaterials*. 2013;34:5907–14.
121. Masand SN, Chen J, Perron IJ, Hammerling BC, Loers G, Schachner M, *et al*. The effect of glycomimetic functionalized collagen on peripheral nerve repair. *Biomaterials*. 2012;33:8353–62.
122. Joung D, Lavoie NS, Guo S-Z, Park SH, Parr AM, McAlpine MC. 3D printed neural regeneration devices. *Adv Funct Mater*. 2020;30:1906237.
123. Zhang JM, Chen YW, Huang YL, Wu WB, Deng XM, Liu HF, *et al*. A 3D-printed self-adhesive bandage with drug release for peripheral nerve repair. *Adv Sci*. 2020;7:2002601.

Downloaded from https://academic.oup.com/burnstrauma/article/doi/10.1093/burnst/tkab011/6271241 by guest on 12 June 2022