



Fabrication Techniques of Nerve Guidance Conduits for Nerve Regeneration

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Neuronal loss and axonal degeneration after spinal cord injury or peripheral injury result in the loss of sensory and motor functions. Nerve regeneration is a complicated and medical challenge that requires suitable guides to bridge nerve injury gaps and restore nerve function. Due to the hostility of the microenvironment in the lesion, multiple conditions should be fulfilled to achieve improved functional recovery. Many nerve conduits have been fabricated using various natural and synthetic polymers. The design and material of the nerve guide conduits were carefully reviewed. A detailed review was conducted on the fabrication method of the nerve guide conduit for nerve regeneration. The typical fabrication methods used to fabricate nerve conduits are dip coating, solvent casting, micropatterning, electrospinning, and additive manufacturing. The advantages and disadvantages of the fabrication methods were reported, and research to overcome these limitations was reviewed. Extensive reviews have focused on the biological functions and in vivo performance of polymeric nerve conduits. In this paper, we emphasize the fabrication method of nerve conduits by polymers and their properties. By learning from the existing candidates, we can advance the strategies for designing novel polymeric systems with better properties for nerve regeneration.

Key Words: Nerve regeneration, nerve guidance conduits, fabrication, spinal cord, peripheral nerve

INTRODUCTION

Nerve injury damages the brain, spinal cord, or peripheral nervous system and induces serious health problems.¹⁻³ A complete injury of the nerve causes the total loss of motor or sensory functions pertaining to the injury site, while an incomplete injury causes the retention of some functions. The loss of neurons and degeneration of axons result in the loss of function. Nerve injury is a debilitating and irreversible injury that leads to complete or incomplete loss of sensory and motor function

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beneath the injury area, depending on the extent of the injury. Such injuries not only cause disabilities for individuals and their families, but also burden health systems and economies with the loss of productivity and high healthcare costs. The average lifetime costs reach a staggering \$1.1-4.6 million per spinal cord injury (SCI) patient, with over 1 million people affected in North America alone.⁴ To understand the complicated interaction of nerve components in tissue-engineered conduits, we need to understand the molecular interactions of normal nerve injury.

SCI is commonly divided into primary and secondary injuries. Primary injury refers to direct mechanical injury to the spinal cord. However, in most clinical situations, secondary injury following the primary injury is more important, and a therapeutic treatment is needed to prevent the propagation of injury.⁵ Primary injuries, such as cell necrosis, axon disruption, and vascular loss, result from a direct impact on the spine and trigger pathophysiological secondary injuries.^{6,7} In addition, secondary injury induces disturbances in ionic homeostasis, hemorrhage, ischemia, glutamate release, cell death, immune response, and oxidative damage.⁸⁻¹¹ Disruption of spinal cord blood vessels induces hemorrhage and ischemia at the SCI

site.¹²⁻¹⁴ In addition, excessive activated chemicals, such as the excitatory neurotransmitters glutamate or aspartate, cause damage to neurons, astrocytes, and oligodendrocytes. As a result, damage to oligodendrocytes leads to axonal demyelination, which impairs nervous system plasticity. Glial scars composed of myelin, cellular debris, astrocytes, oligodendrocytes, and microglia hinder the regeneration of axons toward their synaptic targets.¹⁵⁻¹⁷ For SCI therapy, high doses of methylprednisolone have been used in clinical treatment. However, several studies reported that treatment with methylprednisolone showed weak neurological improvement after SCI.¹⁸

SCI and peripheral nervous injury differ in regenerative capacity after injury. Neurons in the peripheral nervous system are capable of regeneration after injury; however, neurons in the spinal cord are generally not.¹⁹ Schwann cells in the peripheral nervous system, such as degeneration, remyelination, and axonal growth, play a pivotal role in several aspects of nerve repair. Peripheral nerve injury (PNI) represents a clinical and public health problem.²⁰ These cases can potentially lead to lifelong disabilities, although peripheral nerves exhibit the capacity of self-regeneration for less severe injury. Currently, the treatment for PNI is advanced microsurgical end-to-end repair with tensionless epineurial sutures or autologous nerve grafting with end-to-end anastomosis.^{21,22}

A tissue engineering construct and contact-mediated guidance for aligned axon growth across the site of injury to the distal host tissue could potentially allow functional recovery. Various bioengineered nerve grafts have been developed using materials and fabrication methods for nerve regeneration. In tissue engineering, the tubular structure fabricated from natural or synthetic biopolymers for nerve regeneration is called nerve guidance conduits (NGC).²² NGCs were designed considering the mechanical and biochemical factors that can promote nerve regeneration. The role of NGCs is connected to injured nerve endings and provides structural and biochemical support.²³ It promotes nerve regeneration along the conduit and prevents the invasion of the surrounding tissues. An ideal NGC should have a biomimetic architecture, structural features to align the axon of the regenerating nerve, sufficient mechanical properties, and permeability for nutrient delivery, conductivity, flexibility, and biodegradability.²⁴ However, to develop an ideal NGC, a number of studies have been reported on various fronts, including the design, materials, and fabrication methods. In this review, we discuss the fabrication method of nerve guide conduits by tissue engineering for nerve regeneration.

DESIGN AND MATERIALS FOR NGCS

Several types of research on NGC design have been reported. The signs of NGCs can be divided into five categories: 1) hollow, nonporous design, 2) porous design, 3) grooved design,

4) multichannel design, and 5) NGCs with fillers. Hollow or nonporous designs are the simplest hollow conduit designs made of natural or synthetic polymers. Although this design is simple to fabricate, the permeability of oxygen and nutrients is low, so there is a limit to nerve regeneration. The porous design is a conduit design with a porous wall to overcome the disadvantages of the hollow design. This design has a high permeability, which is advantageous for supplying nutrients. However, owing to their high permeability, the surrounding tissues can infiltrate into the inner conduit.

The grooved design is grooved on the inner surface of the conduit. The groove of the inner surfaces is expected to align better with the regeneration of the axons. The multichannel design is a conduit design with intraluminal channels. This design can be loaded with various nerve regeneration factors. NGCs with filler designs are filled with nanofibers or hydrogels in the lumen. This design filled the inside of the conduit with nanofibers and hydrogels to mimic nerve fascicles.

The choice of materials for the fabrication of NGCs for nerve regeneration is a particularly important consideration. The basic requirements for NGC fabrication materials are biocompatibility, biodegradability, and suitable mechanical properties. In addition, nerve guide materials are highly permeable and sufficiently flexible with suitable degradation rates and products to provide guidance for regenerative axons and to minimize swelling and inflammatory responses. Natural and synthetic polymers are the most commonly used materials. The most widely used natural polymers are collagen, gelatin, cellulose, and chitosan. Natural polymers provide a biomimetic and cell-friendly environment, but have low mechanical properties. The most widely used synthetic polymers are polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid, and poly (lactic-co-glycolic acid) (PLGA). Synthetic polymers provide good mechanical properties, but are not biomimetic. A blend of natural and synthetic polymers is often used to overcome these limitations.

FABRICATION METHODS OF NGCS

Several fabrication methods have been employed for the development of NGCs (Tables 1 and 2). The most common NGC fabrication methods are dip coating, solvent casting, freeze-drying, micropatterning, electrospinning, and additive manufacturing (AM) (Fig. 1).

Dip coating

The dip-coating method is a widely used technology for NGC fabrication, as it is the easiest method. The fabrication process of the dip-coating method is as follows. The first step is to prepare the polymer emulsion. This is prepared by dissolving the polymers in solvents. The second step is to dip the round mold in the prepared polymer emulsion. The dipped round mold is

Table 1. Fabrication Techniques of NGC and Other Effective Factors for Nerve Regeneration

Fabrication method	Biomaterial	Added factors	Finding	Reference
Dip coating	Tyrosine-derived polycarbonate terpolymer	Collagen m-HNK	The use of cell-friendly fillers, such as collagen, may limit nerve regeneration	53
Dip coating	Tannic acid/ Poly (N-vinylpyrrolidone)	Mg-based metallic glass	Mg-based metallic glass is a promising candidate for nerve regeneration	54
Dip coating	Polycaprolactone	Graphene	The electrical conductivity of material could be a suitable candidate for use as a versatile system	55
Dip coating	Polyhydroxy-alkanoates		Polyhydroxyalkanoates are indeed highly promising candidates for peripheral nerve regeneration	56
Solvent casting Micro patterning	Chitosan		Micro-structured asymmetrical directional topographies enhanced nerve regeneration	57
Solvent casting	Polycaprolactone Poly (lactic-co-glycolic acid) polypyrrole		The fibrous morphology containing interconnected pore and the hydrophilic surface of composite facilitated cell adhesion and proliferation	58
Solvent casting	Polyglycerol-sebacate/ Polycaprolactone	Graphene	Graphene improved cell survival and cell attachment	59
Freeze-drying	Collagen		Fibrillar NGC with the aligned topographical feature enhanced nerve regeneration	60
Freeze-drying	Collagen	Hyaluronic acid	Biphasic NGC was shown to support neurogenesis and gliogenesis of neural progenitor cells	61
Freeze-drying	Erythropoietin Chitosan		NGC could significantly accelerate nerve healing and improve morphological repair	62
Freeze-drying	Flammulina velutipes		FVC exhibited excellent biocompatibility and effectively promoted cell proliferation and elongation	63

NGC, nerve guidance conduits.

coated with a polymer emulsion. The third step is to remove the round mold from the polymer emulsion and the hardening of the polymer emulsion via evaporation or polymerization. Finally, a conduit structure can be produced by removing the round mold.

Ko, et al.²⁵ fabricated a gelatin conduit using a dip-coating method for peripheral nerve regeneration. The reported NGCs were fabricated using gelatin and bisvinyl sulfonemethyl (BVSM). It has a shape similar to that of a silicone conduit used in clinical practice. The dip-coating procedure was conducted nine times to produce a conduit coated with 400-µm thick gelatin. The NGCs were assessed for neuronal electrophysiology, animal behavior, neuronal connectivity, macrophage infiltration, calcitonin gene-related peptide localization, and expression, as well as the expression levels of nerve regeneration-related proteins. In addition, the number of fluorogold-labeled cells and histological analysis of the gelatin-BVSM nerve conduits was similar to that observed with the clinical use of silicone rubber conduits after 8 weeks of repair.

Lin, et al.²⁶ fabricated a PLGA multilayer conduit using a dip-coating method for nerve regeneration. A PLGA multilayer nerve conduit with an associated biodegradable drug reservoir was designed, fabricated, and evaluated. Moreover, to demonstrate the potential of this device for nerve repair, a series of experiments were performed using the nerve growth factor (NGF). As a result, bioactivity assays of the released NGF

showed that the drug released from the device between the 15th and 20th day could still promote axon growth (76.6–95.7 µm) in chick dorsal root ganglion cells, which is in the range of maximum growth. As mentioned earlier, NGCs fabricated by dip coating can promote nerve regeneration. The advantage of the dip-coating method is its simpler procedure and easy control of the thickness. However, this method cannot create pores for nutrient transport on the wall of the conduit.

Solvent casting

Solvent casting is the cheapest and easiest method to fabricate porous NGC. The fabrication process of the solvent casting method is as follows. The first step is to dissolve the polymer in the solvent. The second step is to fill the mold. Finally, the evaporation of the solvent results in a porous structure. Guo, et al.²⁷ fabricated hollow chitosan conduits using a solvent casting method for peripheral nerve regeneration. A solvent casting procedure was conducted to produce a thickness of 400 µm. The chitosan conduit was then filled with 0, 0.5, or 1.0 mg of simvastatin in Pluronic F-127 hydrogel. Sciatic functional index, electrophysiological assessments, and histological and immunohistochemical assessments were conducted, and it was confirmed that the F-127 hydrogel promoted nerve regeneration. As a result, the chitosan conduit filled with the simvastatin/Pluronic F-127 hydrogel promoted nerve regeneration.

Table 2. Fabrication Method of NGC and Other Effective Factors for Nerve Regeneration

Fabrication method	Biomaterial	Added factors	Finding	Reference
Electrospinning	Poly (lactic-co-glycolic acid) Polylactic acid Polyglycolic acid		The gradient degradable nerve guidance conduit with multilayer structure is designed and fabricated via electrospinning technique	64
Electrospinning	Polycaprolactone	Fe ₃ O ₄ -MNPs MLT	Multilayered composite NGC shows great prospect in long-term nerve regeneration	65
Electrospinning	Polycaprolactone Sodium alginate		Bilayer cylindrical conduit promotes the migration of Schwann cells along the axon	66
Electrospinning	Poly (D, L-lactic acid) Collagen	β-tricalcium phosphate	Composite NGC has a bionic structure, good cellular affinity, and biodegradability, which can overcome the limitation of nerve conduits	67
Electrospinning Micropatterning	Polylactic acid Collagen		Multilevel structure promoted cell infiltration	68
Extrusion-based printing	Alginate		3D print and pattern bi- and tri-layered hollow channel structures improved cellular adhesion	69
Extrusion-based printing Micropatterning	Gelatin	Graphene	The electrical stimuli applied within the 3D gelatin matrix enable enhanced differentiation and paracrine activity, leading to promising nerve regeneration	70
Extrusion-based printing	Polycaprolactone Chitosan	Hydroxyapatite	Multilayered NGC can be functionalized by incorporation of mechanical or biological cues that favor ingrowth, guidance, and correct targeting of axons	71
Digital light processing	Gelatin methacrylate		Multichannel NGCs with different inner diameters were successfully fabricated	72
Digital light processing	Gelatin methacrylate Poly (ethylene glycol) diacrylate	Platelets	3D-printed nerve conduit with live platelets may show potential clinical application in nerve repair	73
Digital light processing Micropatterning	Gelatin methacrylate	XMU-MP-1 nanoparticles	3D-printed self-adhesive NGCs with nanoparticles were fabricated	74
Inkjet 3D printing	Polydopamine Arginylglycylaspartic acid	Graphene	Multilayered porous NGCs were fabricated and demonstrated that graphene-based nanotechnology has great potentials in nerve repair	45
Inkjet 3D printing	Collagen Polycaprolactone	Nanoceria	Multilayered porous NGCs were fabricated and demonstrated compelling evidence for future research in antioxidant nerve conduits	46

NGC, nerve guidance conduits; 3D, three-dimensions.

Fregnan, et al.²⁸ fabricated a chitosan membrane using a solvent casting method for peripheral nerve regeneration. The chitosan membrane was crosslinked with dibasic sodium phosphate (DSP) or γ -glycidoxypropyltrimethoxysilane. The chitosan membrane was rolled and glued with cyanoacrylate glue to obtain a conduit. In addition, evaluation of chitosan/DSP conduits promoted nerve fiber regeneration and functional recovery, leading to an outcome comparable to that of the median nerve repaired by autograft.

The PLGA multilayer conduit was fabricated a solvent casting method for peripheral nerve regeneration.²⁹ The PLGA-based multilayer nerve conduit has a reservoir for local delivery of NGF. This nerve guide can release NGF for extended periods of time and enhance axon growth in vitro and in vivo, and has the potential to improve nerve regeneration following PNI. Therefore, the advantage of the solvent casting method is that it is a relatively simple procedure, and the porosity and pore size can be controlled. However, the disadvantage of this

method is the use of highly toxic solvents, poor pore interconnectivity, and irregularly shaped pores. To overcome the limitations of the solvent casting technique, other techniques that do not use solvents, such as salt leaching, freeze-drying, and gas foaming, are being researched.

Freeze-drying

The freeze-drying method is used to fabricate the polymeric porous conduits. The fabrication process consists of two steps. The first step is the freezing step, which cools the polymer solution to a certain temperature. During freezing, interstitial spaces are formed as the solvent forms ice crystals, and polymer molecules aggregate into the interstitial spaces. Then, the solvent removes the phase, in which a low pressure is applied, which is lower than the equilibrium vapor pressure of the frozen solvent. The solvent is completely sublimated to fabricate a dry interconnected porous polymer structure.

Yao, et al.³⁰ and Cui, et al.³¹ fabricated collagen nerve con-

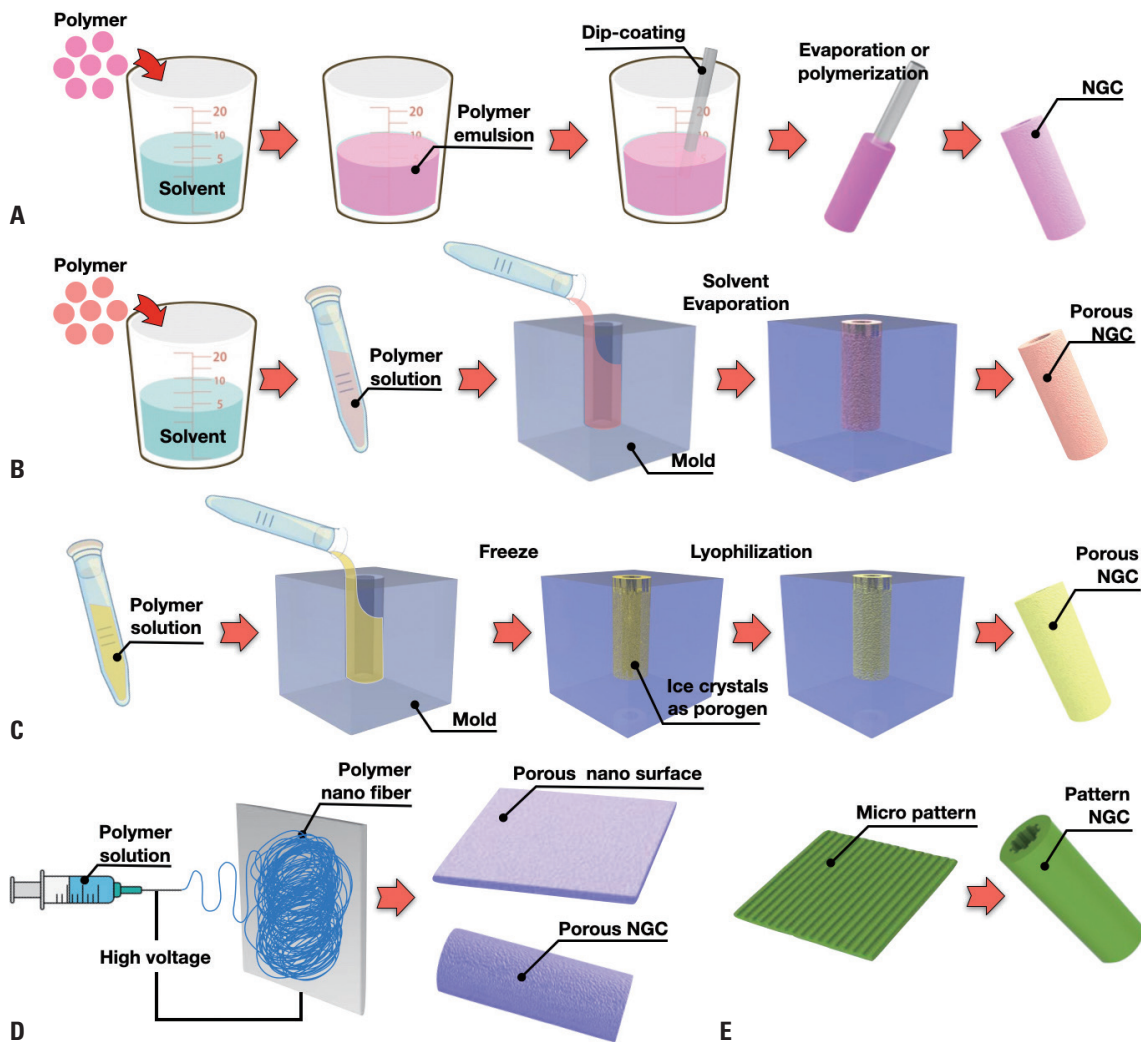


Fig. 1. Common techniques used for nerve guide conduit fabrication. A: Dip-coating. B: Solvent casting. C: Freeze drying. D: Electrospinning. E: Micro-patterning. NGC, nerve guidance conduits.

duits using the freeze-drying method. The collagen nerve conduit was created by rolling the collagen membrane. The diameter of the cylindrical conduit was approximately 5 mm, and the tube wall thickness was 60 μm . Long-term follow-up evaluation demonstrated that NGC allowed functional and morphological nerve regeneration. Conclusion: functional assessment of the conduit confirmed that when NGF was loaded onto a longitudinally oriented collagen conduit, it promoted a better recovery of regenerated axons compared to longitudinally oriented collagen conduit alone. Consequently, the advantage of the freeze-drying method is that it can achieve a highly porous structure. Therefore, the permeability of the conduit is high, and the transport of nutrients and oxygen is good, which promotes nerve regeneration. However, in this method, the size and shape of the pores were relatively irregular compared to those of solvent casting.

Electrospinning

Electrospinning is the most widely used and reported tech-

nique among NGC fabrication methods. Electrospinning is a method of extracting nanofibers by applying an electric field (high voltage) to a polymer solution. The first step of electrospinning is the extrusion of the polymer solution through a nozzle tip with a high applied voltage. The extrusion solution is not dropped by gravity, and is clumped at the nozzle tip by surface tension. At this time, charges are collected on the surface of the solution by a high voltage. In addition, a repulsive force is generated between the charges, the polymer solution overcoming the surface tension, and ejecting. The ejected solution moves to the collector according to the gradient of the electric field. While the ejected solution is supplied by an electric field, the solvents in the ejected solution evaporate, thereby forming nanofibers through secondary fission.

To describe the reported studies, Xue, et al.³² fabricated electrospinning silk fibroin (SF)-based nerve conduits. A simple electrospinning SF-conduit was collected on a rotating stainless-steel rod (diameter, 5 mm) at a rotating speed of 500 rpm. In this study, functional, histological, and morphometric

analyses were conducted, and it was confirmed that SF-based conduits achieved satisfactory regenerative outcomes. Wang, et al.³³ fabricated an electrospinning SF/poly (L-lactide-co-caprolactone) [SF/P (LLA-CL)]-based nanofiber membranes. A nerve conduit was fabricated by reeling the membrane onto a stainless-steel bar with a diameter of 1.4 mm. The results revealed that the SF/P (LLA-CL) nerve conduit enhanced peripheral nerve regeneration by improving angiogenesis within the conduit. Therefore, the electrospinning method can be used to fabricate nanofiber architectures, and can also be used widely in materials. In addition, nanofiber architecture is similar to the extracellular matrix, and can positively affect cell attachment and migration compared to micro-architecture. The drawback of electrospinning is its low scalability and low reproducibility, as nanofibers are stacked randomly.

Micropatterning

The micropatterning technique is used to fabricate NGCs with various inner designs for axonal alignment. The first process of the micropatterning technique is the fabrication of a micropattern membrane. After fabrication of the micropattern membrane, the micropattern membrane was rolled to fabricate the conduit. To describe the reported studies, Rutkowski, et al.³⁴ fabricated a multilayer NGC using novel transfer techniques and the salt-leaching method. The mold for the micropattern was fabricated using silicon and quartz dies and the reactive ion etching method. The micropattern consisted of a width of 10 μm , depth of 4.3 μm , spacing of 10 μm , and length of 4 cm, and was fabricated using poly (D, L-lactic acid) (PDLLA). The porous layer was fabricated using the salt-leaching method and PDLLA. Based on qualitative observations as well as quantitative measurements using the walking track analysis, micropatterned conduits were significantly enhanced, which was confirmed.

Ni, et al.³⁵ fabricated a microporous/micropatterned NGC using molding and phase separation methods. The micropattern consisted of a width of 20 μm , depth of 3 μm , and spacing of 20 μm , and was fabricated using PLA. Axon regeneration and functional recovery of the PLA conduit were evaluated by histology, walking track analysis, and electrophysiology. In addition,

PLA conduits grafted with chitosan-nano Au and FGF1 after plasma activation had the greatest regeneration capacity and functional recovery in the experimental animals. Consequently, the advantage of micropatterning is that it can align axons by morphological factors inside the NGC. However, the micropatterning technique should be used to fabricate master molds and multistep processes.

Additive manufacturing

AM is a three-dimensions (3D) printing processing method that manufactures 3D structures using layer-by-layer deposition materials. AM has various manufacturing methods, such as stereolithography (SLA), selective laser sintering, fused deposition modeling (FDM), inkjet printing, and 3D plotting (Fig. 2). The advantage of AM is that the degree of freedom of the shape is high, so the morphological characteristics of the structure can be controlled and the reproducibility is high. However, depending on the AM method, the materials that can be used are limited, the shape resolution is low, and a supporter is required to fabricate a complex structure.

Extrusion-based printing

Extrusion-based printing is a method of manufacturing 3D structures by extruding and depositing materials using pneumatics, pistons, screws, and nozzles. Extrusion-based printing is divided into FDM, precision extrusion deposition, and 3D plotting, depending on the extrusion method. The advantage of extrusion-based printing is that it can achieve one-step printing of NGCs containing various composite materials that are difficult to obtain by traditional manufacturing methods.³⁶ However, the limitations of extrusion-based printing are that the efficiency and accuracy of printing are low due to using a nozzle, which is prone to blockage.³⁷ To describe the reported studies, Cui, et al.³⁸ fabricated multilayer polyurethane (PU)/collagen NGC using a modified FDM method. The multilayer NGC was composed of a PU outer layer containing a microporous structure and a collagen inner layer.

The indirect biological-printing method of extrusion-based printing has also been used to produce NGCs. Hu, et al.³⁹ fabricated cryoGelMA NGCs using an indirect biological-printing

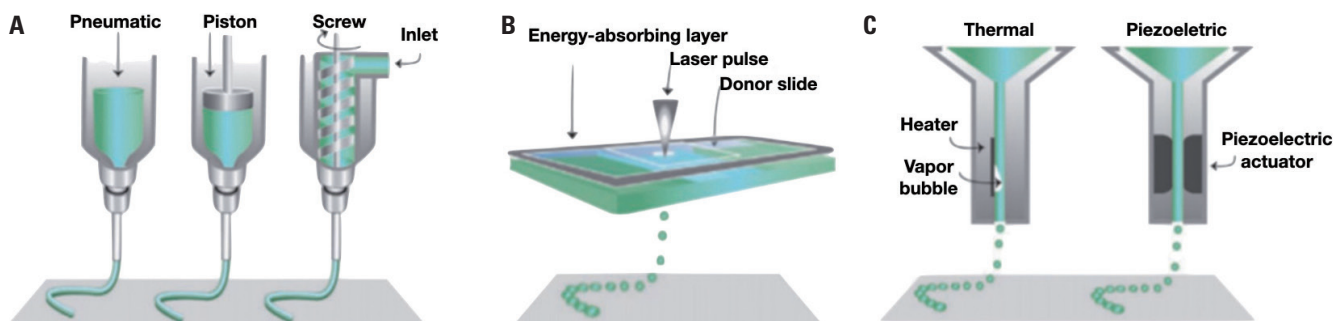


Fig. 2. Additive manufacturing techniques used for nerve guide conduit fabrication. A: Extrusion-based printing. B: Stereolithography. C: Inkjet three-dimensions printing.

method. The mold structures were fabricated using a 3D printing technique. Assisted by the 3D printed mold, cryoGelMA NGCs with intricate architectures can be obtained. In this study, the assessment of attachment, proliferation, and upregulation of the expression of their neurotrophic factor mRNA of the cryo-GelMA NGCs was conducted. Moreover, the cryoGelMa NGCs to be close to those of the autografts in terms of functional and histological assessment were confirmed.

Stereolithography and digital light processing

SLA is a 3D printing technique that uses a laser light source and a photocurable polymer to fabricate a 3D structure. The first step of the SLA process is to add a photosensitive resin to the vat and flatten it. Subsequently, ultraviolet light (UV)-wavelength laser irradiation of photosensitive resin causes polymerization of the resin, and a single cured layer is obtained. The work platform is then filled with a new photosensitive resin layer to continue the curing reaction of the next layer. This process is repeated layer-by-layer to obtain a 3D structure.

The advantages of SLA techniques are higher accuracy and reproducibility than those of extrusion-based printing techniques. However, this method can only be used with photosensitive resin, and photo initiators should be added to confirm the cytotoxicity of the material. To describe the reported studies, Singh, et al.⁴⁰ fabricated biodegradable poly (glycerol sebacate methacrylate) (PGSm) NGCs using the SLA technique. PGSm was synthesized, and its degradation rate and mechanical properties were assessed. The fabricated NGC inner diameter was 1 mm, and the wall thickness was 0.35 mm. In vivo results in a mouse common fibular nerve injury model confirmed that repair levels of spinal cord glial activation were equivalent to those seen after graft repair. Singh, et al.⁴¹ fabricated a multichannel NGC using the SLA technique and PCL. NGC was filled with NGFs for peripheral nerve regeneration. This demonstrated the application of the NGF-filled NGCs in enhanced and successful regeneration of a critically injured rat sciatic nerve in comparison to random cryogel-filled NGCs, multichannel and clinically preferred hollow conduits, and gold standard autografts.

Digital light processing (DLP) is a 3D printing technique similar to SLA. DLP is also used as a UV light source for the polymerization of photopolymers. The greatest advantage of DLP is that it cures a single layer simultaneously, thus fabricating a 3D structure much faster. To describe the reported studies, Zhu, et al.⁴² fabricated various conduits (hollow conduit, multiple microchannel conduit, branched conduit) for human facial nerve repair. The hollow NGC was designed with a wall thickness of 200 μm and a length of 4 mm. The multiple microchannel conduit was incorporated into four microchannels (400 μm in diameter and 4 mm in length) to guide longitudinal nerve regeneration. The branched conduit was designed to integrate two hollow tubular sleeves continuously. Additionally, the materials were synthesized using polyethylene glycol diacrylate,

gelatin-methacryloyl (GelMA), and LAP. Tao, et al.⁴³ fabricated functional nanoparticle-enhanced conduits for effective nerve repair. The conduit consisted of GelMA hydrogels with drug-loaded poly (ethylene glycol)-poly (3-caprolactone) nanoparticles. However, similar to the SLA method, this method can only use photosensitive resin, and photo initiators must be added; it is necessary to confirm the cytotoxicity of the material.

Inkjet 3D printing

Inkjet 3D printing is a 3D printing technique that uses photocurable binders and powder materials. The first step of the inkjet 3D printing process is to planarize the powder materials. Subsequently, the photocurable binder is sprayed and cured to form one layer. The work platform is then filled with new powder materials. This process is repeated layer-by-layer to obtain a 3D structure. The inkjet 3D printing method is composed of continuous inkjet printing and drop-on-demand inkjet printing according to inkjet techniques.

Drop-on-demand inkjet printing is widely applied in bioprinting owing to its unique advantages and suitability for the delivery of biomaterials.⁴⁴ The drop-on-demand inkjet 3D printing method is divided according to the droplet production method. The thermal inkjet 3D printing technique produces droplets according to the local heat of the ink chamber using a heat actuator. The local heat of the ink chamber generates a heat bubble, and the droplets are ejected by the expansion of the heat bubble. The advantage of the thermal inkjet 3D printing method is its fast-printing speed and low equipment cost. However, droplet ejection maintenance is difficult because the droplets are ejected by heat bubbles.

The piezoelectric inkjet 3D printing technique produces droplets according to the piezoelectric actuator. The piezoelectric actuator changes the chamber wall and volume according to the voltage pulse, and hence ejects a droplet. The electrostatic inkjet 3D printing technique produces droplets according to the pressure plate and electrode plate under the action of static electricity. The volume of the ink chamber is reduced by a pressure plate, and the ink is squeezed out as droplets. The electrohydrodynamic jet 3D printing technique produces droplets through an electric field.

The advantages of electrohydrodynamic jet printing are its high resolution and printing capability. To describe the reported studies, Qian, et al.⁴⁵ fabricated multilayered porous NGC using inkjet 3D printing and the layer-by-layer casting method. The NGC consisted of single-layered graphene, multilayered graphene, and PCL.

Qian, et al.⁴⁶ fabricated multilayered porous NGC using inkjet 3D printing and a layer-by-layer casting method. The NGC consisted of collagen, nanoceria, and PCL. This NGC successfully improved Schwann cell proliferation, adhesion, and neural expression.

MULTIPLICATIVE NGCs

As mentioned above, various fabrication techniques have been developed and applied to fabricate an ideal NGC. These fabrication techniques can determine the morphological factors of NGC and have various effects on nerve regeneration. However, there is a limit to nerve regeneration with NGC alone. To overcome this limitation, multiplicative NGCs have been developed using various other factors for nerve regeneration. Other factors used in tissue engineering for nerve regeneration include growth factors and nutrients. Some growth factors that have been used in nerve regeneration include NGF, glial cell-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), neurotrophin 3, vascular endothelial growth factor (VEGF), and insulin-like growth factor 1. To describe the reported studies, Oh, et al.⁴⁷ fabricated NGC with an NGF gradient along the longitudinal direction by rolling a porous PCL membrane with an NGF concentration gradient. They reported that NGF promoted nerve regeneration, and that the concentration gradient of NGF could be a promising strategy for nerve regeneration.

Liu, et al.⁴⁸ designed and fabricated multilayered aligned fiber scaffolds by combining emulsion electrospinning, sequential electrospinning, and high-speed electrospinning to modulate the release behavior of GDNF and NGF. Su, et al.⁴⁹ fabricated a composite NGC containing slow-released BDNF and evaluated its therapeutic effects on peripheral nerve defects. The BDNF-loaded composite conduit was fabricated using the solvent casting method. Xia, et al.⁵⁰ fabricated a VEGF and NGF-loaded nanofibrous NGC using the emulsion electrospinning method.

Another factor in tissue engineering is the addition of nanoparticles, which can enhance nerve regeneration. Many studies have reported that gold nanoparticles, silver nanoparticles, and iron oxide nanoparticles have shown immense potential for nerve regeneration.

Jahromi, et al.⁵¹ developed a novel strategy for nerve regeneration using gold nanoparticles (AuNPs) and BDNF-encapsulated chitosan in laminin-coated nanofibers of PLGA conduit. Gold nanoparticles and BDNF-encapsulated nanofiber NGC were fabricated by electrospinning. Dolkhani, et al.⁵² fabricated a chitosan-selenium biodegradable nanocomposite conduit.

CONCLUSIONS

In this review, we briefly describe nerve regeneration in tissue engineering. In addition, we present the ideal requirements for NGC, which is essential for nerve regeneration. The materials and designs used in NGC fabrication for nerve regeneration are briefly described; in particular, the NGC fabrication processes are described in detail, and the advantages and disadvantages

of each fabrication method are introduced. The reviewed NGC fabrication methods were dip coating, solvent casting, freeze-drying, electrospinning, micropatterning, and additive manufacturing, and representative research cases are described. Moreover, multiplicative NGC fabrication, which is being researched to overcome the limitations of each fabrication method, is also described. However, the reported NGCs do not completely regenerate nerves and have difficulties in clinical application. Therefore, further research on the fabrication of an ideal NGC for complete nerve regeneration should be conducted.

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AUTHOR CONTRIBUTIONS

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