ACCSCIENCE PUBLISHING

International Journal of Bioprinting

REVIEW ARTICLE

Three-dimensional bioprinting technologies and biomaterials for nerve guidance conduits: A review

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Abstract

Repairing long peripheral nerve gap injuries and reconstructing corresponding functions remain two major challenges in regenerative medicine. The application of nerve conduits, constructed via neural tissue engineering (NTE) strategies, has emerged as a prominent research focus and an essential tool for nerve repair. Among NTE technologies, additive manufacturing (AM), especially bioprinting, represents one of the most promising fabrication approaches for neural conduits. This review systematically analyzes the current research progress on peripheral nerve conduit fabrication, particularly emphasizing how different conduit structures, biomaterials, and AM techniques synergistically influence nerve regeneration outcomes. The review also summarizes the principles and recommendations for selecting appropriate nerve conduit structures for different defect lengths and injury stages, providing a theoretical basis for the design and practical application of conduit structures. Additionally, it focuses on the role of advanced bioprinting technologies in enhancing conduit complexity, cell guidance, and functional recovery. Furthermore, this review highlights emerging trends and discusses critical future directions for integrating structure design, material selection, and printing strategies toward the next generation of nerve conduits. This review aims to provide a comprehensive perspective for advancing peripheral nerve repair by bridging biomaterial engineering, manufacturing innovations, and regenerative medicine needs.

***Keywords*:** Additive manufacturing; Biofabrication; Nerve conduit; Neural tissue engineering

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Citation: Zhuang Y, Seiti M, Ferraris E. Three-dimensional bioprinting technologies and biomaterials for nerve guidance conduits: A review. *Int J Bioprint*. 2025;11(4):32-65. doi: 10.36922/IJB025140120

Received: April 2, 2025
Revised: May 16, 2025
Accepted: May 26, 2025
Published Online: June 6, 2025

1. Introduction

Peripheral nerves are characterized by a complex structure and wide distribution, making peripheral nerve injury (PNI) a very common injury in the human body.[1] Once the peripheral nerves are damaged and the nerve conduction pathways are interrupted, the respective target organs are impaired, and bodily functions are hampered. Injured peripheral nerves can self-regenerate under specific microenvironmental conditions,[2] but this repair capacity is limited. Understanding the structural organization and regenerative mechanisms of peripheral nerves is important for elucidating the post-

injury repair process and formulating effective intervention strategies. Nerves are composed of numerous axons that are wrapped and supported by connective tissues, including the endoneurium, perineurium, and epineurium. The anatomy of a healthy peripheral nerve is shown in Figure 1.

The etiology of traumatic PNI usually includes penetrating trauma, traction and compression, ischemia, electrocution, and vibratory injuries. In 1943, British neurosurgeon Herbert Seddon[3] classified nerve injuries into three categories based on the degree of damage to the neural structures: neurapraxia, axonotmesis, and neurotmesis. This is one of the most classic and basic grading systems for nerve injuries. In 1951, Sunderland[4] further refined this classification by dividing PNIs into five classes according to the depth of injury and the degree of structural damage, as shown in Table 1. This classification system helps to describe the damage of nerve structures (e.g., axons, myelin sheaths, nerve endothelium, fasciculus, and ependyma) more accurately from Grade I to V with increasing structural damage and provides a theoretical basis for the subsequent design and selection of different nerve conduits.

It is also important to note that various types of PNI exhibit significant differences in mechanisms, regenerative potential, and clinical manifestations. Studies have demonstrated that peripheral nerve defects with small defects and short gap lengths (usually less than 5 mm) may heal spontaneously under specific conditions.[5] In contrast, when the nerve defects are larger, and the gap length is longer, it is difficult for regenerated nerve axons to accurately bridge the proximal-distal ends of the defective nerves.[6] Consequently, the nerve's self-repair ability is limited. More severe nerve deficits often result in lifelong disability. Therefore, the repair of long-gap peripheral nerve defects and the reconstruction of the corresponding function are major challenges in the field of regenerative medicine.[7]

Autologous nerve grafting is considered the gold standard for the treatment of PNI.[8,9] However, inherent shortcomings, such as limited donor availability and susceptibility to neuroma formation, have prevented autologous nerve grafts from being widely used in clinical practice.[10] Neuroma is a non-malignant neural tissue mass, usually formed due to abnormal proliferation and disordered arrangement of regenerating axons in the absence of effective guidance after PNI.[11] It not only hinders normal nerve regeneration and interferes with the correct connection between axons and target tissues but also may trigger persistent neuropathic pain, which severely affects the patient's sensory and motor functions and even leads to functional reconstruction failure. Therefore, effective prevention of neuroma formation during nerve repair is one of the key objectives to ensure the quality of regeneration.

In recent years, the development of biomedicine and cross-disciplinary interventions, such as biomaterials, biotechnology, and tissue engineering, have provided new solutions for repairing defective nerves. Among

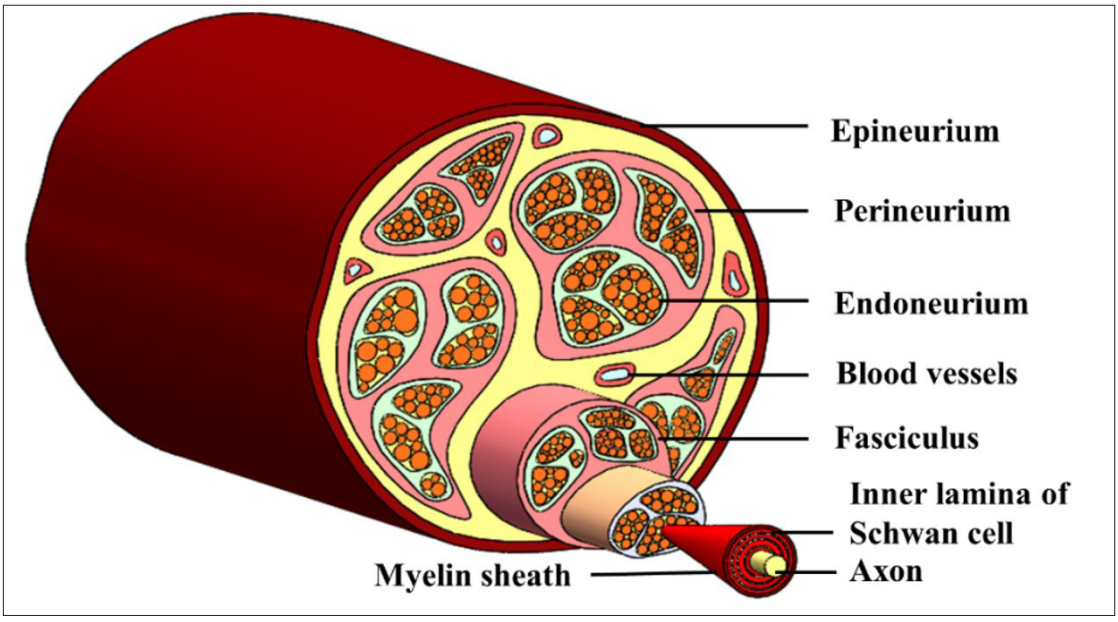

Figure 1. Anatomy of a peripheral nerve in a healthy state.

Table 1. Sunderland's classification of peripheral nerve injuries

Grade	Structural damage	Regeneration potential
I	Local myelin disruption only; axon and connective tissue intact	Complete; recovers in days to weeks
II	Axonal disruption; endoneurium, perineurium, and epineurium intact	Complete; good outcome, about 4–6 weeks
III	Damage to axon, myelin, and endoneurium; perineurium and epineurium intact.	Partial; risk of misdirection
IV	Damage to all structures except epineurium (axon, myelin, endo-, and perineurium)	Poor; surgical repair often required
V	Complete nerve transection, including epineurium	None; requires surgical repair

them, neural tube scaffolds constructed via neural tissue engineering (NTE) approaches have become the main research direction to guide the regeneration of defective nerves.[12] The core of NTE lies in using functionalized biomaterials to construct NTE scaffolds that mimic the natural structure of peripheral nerves, provide a suitable microenvironment for nerve regeneration, and ultimately promote nerve repair.[13] Currently, NTE scaffolds are mostly tubular scaffolds with diameters and lengths matching those of the defective nerves, also known as nerve guidance conduits (NGCs). Studies have shown that such nerve conduits have many advantages in defective nerve repair by serving as: (i) a three-dimensional (3D) specific structure as a suitable microenvironment for migration, proliferation, and functionalization of neural cells, (ii) contact guidance for the directional growth of axons, which in turn improves the accuracy of nerve alignment, and (iii) a sufficient mechanical support for the regenerating nerve fibers, reducing the tension of the surgical suture opening and preventing the growth of scar tissue. Therefore, these advantages make artificial nerve conduits-guided nerve regeneration an ongoing significant research direction.[14]

The main role of the nerve conduit is to bridge injured nerves, provide space and nutrition for axonal growth, and serve as the interface for cell transplantation seeding to treat nerve injuries—guiding cell growth, proliferation, differentiation, migration, apoptosis, and eventually promoting nerve regeneration. Peripheral nerve regeneration is specifically divided into five stages[15] in the presence of nerve conduits, as illustrated in Figure 2: (i) the fluid phase, (ii) the stromal phase, (iii) the cell migration phase, (iv) the axon repair phase, and (v) the myelin formation phase. The fluid phase is within 24 h after implantation. Plasma effluent produced by proximal and distal nerve stumps fills the conduit, leading to the accumulation of neuro-affective factors and extracellular matrix (ECM) molecules. The stromal phase occurs 1–3 days after implantation. ECM precursor molecules emerge from the proximal end and form decellularized fibrin cords between the distal and proximal ends. The cell migration phase occurs 4–7 days after implantation. Schwann cells (SCs), endothelial cells, and fibroblasts migrate from proximal to distal ends, aligning and proliferating along the fibrin cords to form biologic tissue cords, i.e., Bungner bands. The axon repair phase occurs within the 8th to 14th day after implantation. Biological tissue cords provide nutrients and topographical lines for axon repair, and regenerating axon buds reach the distal end using biological tissue cords under the guidance of growth cones. Lastly, the myelin formation phase occurs 15–28 days after implantation, during which SCs transform into a myelin phenotype and form mature myelin with regenerating axons. This is followed by a 1–3-month functional recovery period to restore nerve conduction and tissue integration.

Accordingly, an ideal NGC should meet the following requirements: (i) possesses bioactivity to guide axon growth from proximal to distal stumps, avoiding the formation of neuromas,[16] (ii) has mechanical properties similar to those of human nerve tissue and with enough flexibility to avoid compression of the nerve tissue (tensile strength from 3.39 to 35.93 N and elastic modulus between 8 and 16 MPa), (iii) has good biocompatibility and non-cytotoxicity when in contact with neural cells, without immune reaction,[17] (iv) possess controlled degradation rate to match the one of peripheral nerve regeneration,[18] so that the ideal NGC will gradually break down as the nerve heals, eliminating the need for surgical removal, and (v) has a suitable porous structure, which helps to block scar tissue that hinders nerve regeneration, while allowing essential nutrients and signals to pass through[19] and providing a healthy environment for cellular communication and axonal growth. The basic requirements of these properties are mainly determined by the material and structure of the neural scaffold.[20] Data for this review were obtained from Web of Science, PubMed, and Scopus databases to ensure coverage of high-quality research in the field. To search for studies related to 3D-printed nerve conduits, a combination of the keywords "3D printing" or "3D bioprinting" and "nerve conduit" or "nerve guidance conduit" or "neural regeneration" was used. The search covered 1991–2023

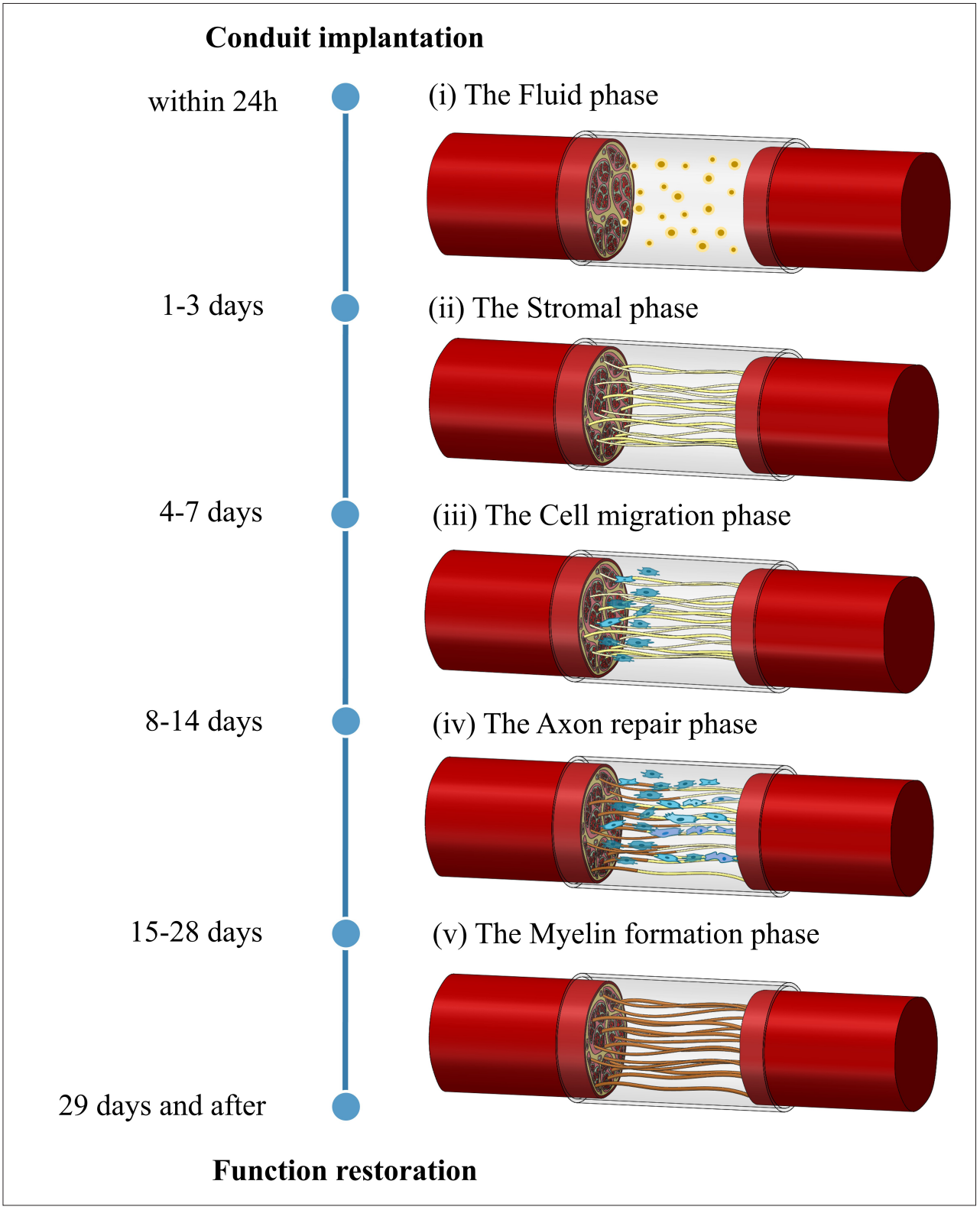

Figure 2. Five stages of peripheral nerve regeneration in the presence of a nerve conduit.

and filtered for topic abstracts and keywords. The statistical data is shown in Figure 3. The statistics include original research, reviews, and conference papers. As shown in Figure 3, research on nerve conduits has increased steadily since the 1990s, with a current focus on the development and study of ideal biomaterials and technologies for nerve conduit biomanufacturing.

Traditional techniques for the preparation of nerve conduits mainly include solvent casting,[21] phase separation,[22] gas foaming,[23] electrospinning,[24] and freeze-drying.[25] These techniques have inherent limitations, such as poor repeatability, low resolution, and limited control of the manufacturable shape, which greatly limit the structure and application of nerve conduits.[26] Additive manufacturing (AM) can effectively overcome (some of) the limitations of these traditional technologies.[27] AM (commonly known as 3D printing) is a manufacturing approach that allows the fabrication of constructs through layer-by-layer (LBL) deposition of materials starting from a digital model file,[28] which is widely used in aerospace,[29] automotive manufacturing,[30] healthcare,[31] construction,[32] textile, and other fields.[33] AM enables the fabrication of complex parts, including scaffolds, lattice structures, and customized patterns, in a wide range of materials, including biopolymers and hydrogels, enabling rapid prototyping of biological conduits with variable designs[34] and providing new solutions for the fabrication of neural conduits.

Since 2011, 3D bioprinting has been increasingly applied to NGC research (Figure 3B). Biofabrication is defined as "the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates, such as micro-tissues, or hybrid cell-material constructs, through bioprinting or bioassembly, and subsequent tissue maturation process".[35(p.5)] This definition includes the fabrication of scaffolds with hierarchical structural properties or smart-surface properties within the realm of bioprinting.[36] The advent of 3D bioprinting has led to more solutions for nerve conduits. Although various biomaterials and preparation strategies have been widely investigated, there are still challenges in achieving good biocompatibility, appropriate mechanical properties, and effective nerve regeneration at the same time. Meanwhile, emerging technologies such as 3D bioprinting offer unprecedented possibilities for constructing NGCs with fine structures, adjustable functions, and individualized features. However, their synergistic integration with applicable biomaterials and bioactive factors is still in its infancy. Therefore, a review of the design principles of nerve conduits, the selection of biomaterials, and the latest advances in 3D bioprinting technologies are needed to identify the current critical issues and provide guidance for future research in this area. Hence, this review will discuss the application of bioprinting technology in the 3D biofabrication of neural conduits, with a focus on its research progress and challenges, specifically divided into three main sections: (i) a discussion of the advantages and limitations of various nerve conduit structures, which provides guidance on selecting appropriate designs under different injury conditions, (ii) introduction of the main biomaterials used in nerve conduit fabrication and their applications in peripheral nerve regeneration, and (iii) summary of current 3D bioprinting technologies for nerve conduits along with relevant fabrication strategies. Finally, the review highlights the key challenges in translating nerve

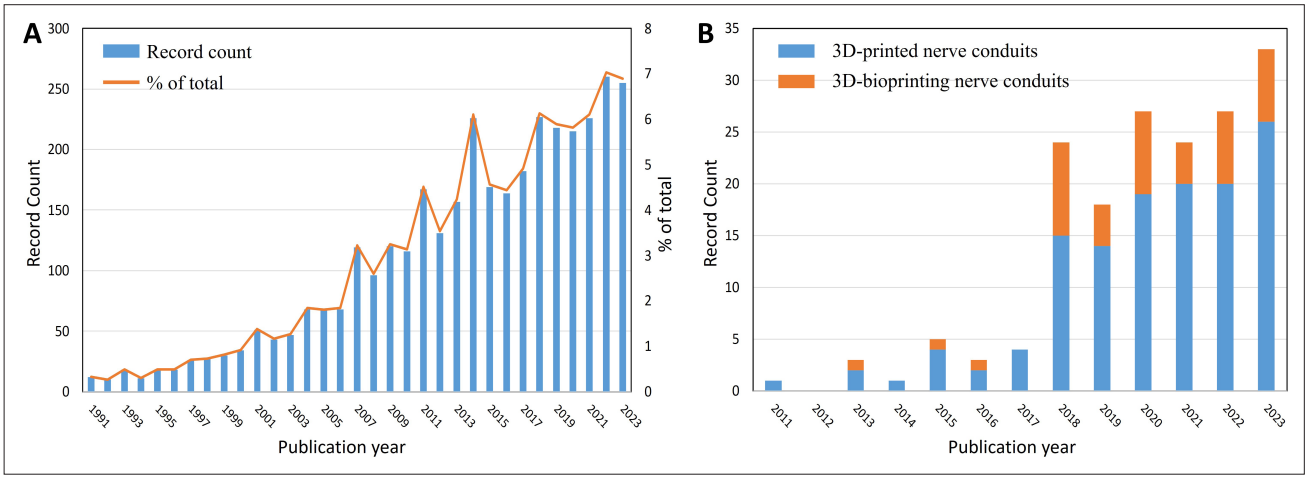

Figure 3. Publication trends in nerve conduit research and 3D printing applications (1991–2023). (A) Number of nerve conduit papers published per year from 1991 to 2023. (B) Number of three-dimensional (3D)-printed nerve conduits and 3D bioprinting nerve conduits papers published per year from 2011 to 2023.

conduits from laboratory research to commercialization and clinical applications and outlines potential future research directions and development trends.

2. Design of a nerve conduit

An ideal nerve conduit should have a rational structural design to provide guidance, support, and a nutrient-rich environment for axonal regeneration. With the advancement of biomaterial science and manufacturing technology, researchers have developed a variety of structural conduits based on the morphological and functional characteristics of natural nerves, such as hollow conduits, multi-channel conduits, porous conduits, conduits with surface microstructures, and bifurcated conduits, as shown in Figure 4. Each structure provides specific solutions for different barriers to nerve regeneration with its own adaptation scenarios and limitations. This chapter systematically introduces the design concepts, key research advances, and experimental performances of different structural nerve conduits.

2.1. Hollow nerve conduit

In preliminary scientific cases, neural conduits were prepared as simple hollow cylinders, commonly known as hollow nerve conduits. This type of conduit has a simple structure that is easily fabricated. It also promotes the formation of fibrin networks during blood coagulation, the migration of various cells (e.g., SCs, endothelial cells, fibroblasts, etc.), and the accumulation of neurotrophic growth factors in the surrounding tissue. This structure is capable of reducing neuromas and scarring and is suitable for repairing short nerve gaps. The first hollow bone bridge for a nerve defect was attempted in a dog model in 1881; however, the results were unsatisfactory.[37] In 1982, Lundborg et al.[38] attempted to bridge a 6 mm nerve defect in rats with a hollow and clear silicone conduit, demonstrating that axonal regeneration begins with the formation of a fibrin clot between the two nerve stumps within the empty conduit, which is subsequently invaded by capillaries, axons, and non-neuronal cells, including SCs. Although the extent and mechanisms by which stump scaffolding promotes cross-gap regeneration were not fully elucidated, the study increased the interest in understanding nerve regeneration. In 1983, Williams et al.[39] further investigated the process of peripheral nerve repair using neural stem cells (NSCs) and impermeable hollow silica nerve conduits by bridging a 10 mm rat sciatic nerve defect. They observed axonal growth reaching the distal stump after 3 weeks.

Although such an NGC structure has been a popular choice for many studies, it presents many drawbacks, such as the lack of morphological and biochemical cues necessary for directional nerve growth,[40] and a very limited effect on the repair of PNI. To better mimic the bundle structure of peripheral nerves, attempts have been made to add fillers, such as fibers, gels, and sponges, as internal filler matrices to reduce the lack of cues required to guide the directional growth of nerves in the hollow conduits and to provide exogenous support for the attachment, migration, and proliferation of SCs. Qin et al.[41] filled the artificial NGC with microfilaments of 80–120 μm in diameter and injected nerve growth factor (NGF) into the conduit to repair sciatic nerve defects in rats. As a result, the number of regenerated axons and myelin maturation were close to those of control autologous nerve grafts. Later,

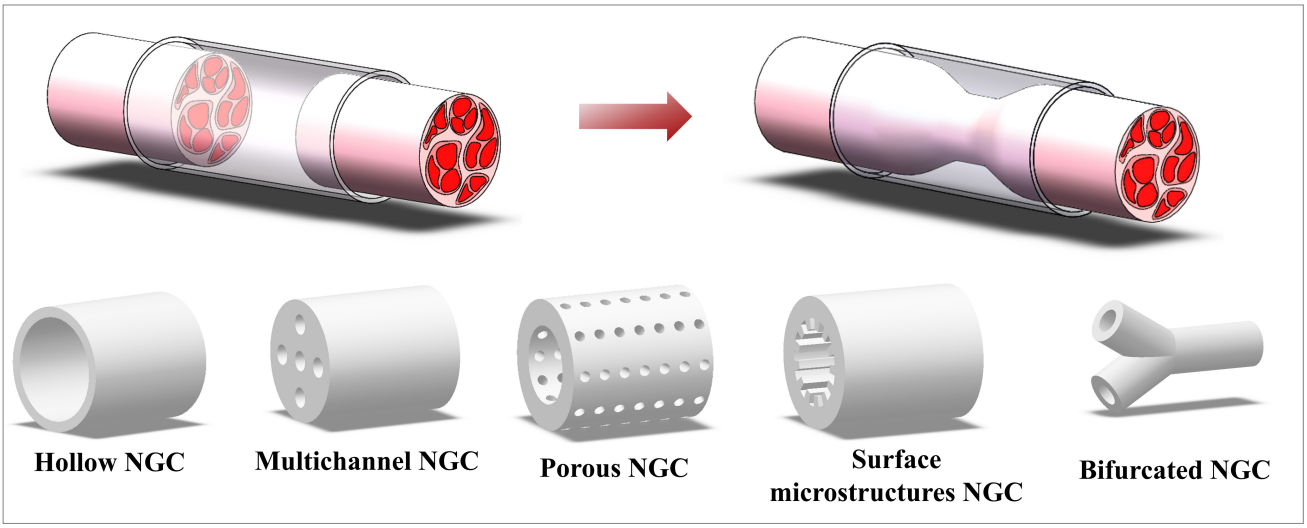

Figure 4. Schematic diagram of various structures of current nerve guidance conduits (NGCs).

Lundborg et al.[42] filled eight longitudinal polyamide fibers into a silicone NGC for the repair of a 15 mm sciatic nerve defect in rats for the first time. Results showed that nerve regeneration was significantly improved compared with that of hollow silicone nerve conduits due to the presence of intraluminal fillers. However, a densely packed lumen matrix was found to hinder axonal regeneration and nerve cell migration. Several studies have demonstrated that the addition of fillers to hollow conduits can compensate for defects and promote nerve repair, but the type, structure, and density of fillers still need further research.

2.2. Multi-channel nerve conduit

Studies have generally shown that multi-channel nerve conduits are more conducive to promoting nerve repair than hollow nerve conduits. First, the longitudinally aligned lumens in multi-channel conduits can act as microtubules with a large surface area required for synthesizing basement membranes, which has a significant positive effect on the attachment, proliferation, and migration of SCs. Moreover, the multiple longitudinal lumens within the nerve conduits reduce axon dispersion and promote the longitudinal extension of axons. This longitudinal arrangement also reduces the rate of axonal misrouting when connecting to distal nerve stumps. However, studies also demonstrated that additional internal structures can affect NGC properties, such as permeability, mechanics, and degradation.[43] In addition, they are also structurally complex and difficult to fabricate.

Over the past few decades, researchers have explored various approaches and fabrication methods to develop microchannels within nerve conduits, which can facilitate nerve repair. In 2004, Moore et al.[44] investigated injection molding and rapid solvent evaporation to fabricate nerve conduits with intraluminal channels. The *in vivo* study on transected adult rat spinal cords further confirmed the presence of regenerating axons 1 month after surgery. Lee et al.[45] also performed an *in vivo* study using arginine–glycine–aspartate (RGD)-functionalized multi-channel nerve conduits in a 10-mm sciatic nerve transection rat model. The results showed that the axon density in the conduit was significantly higher than that in the polycaprolactone (PCL) group after 8 weeks, and the cross-sectional area of gastrocnemius muscle fibers was restored to 80% of that of the healthy control, suggesting that the conduit is close to the autograft in terms of structure, function, and muscle reinnervation. Alternatively, Wang et al.[46] used electrostatic spinning to prepare a multi-channel NGC with directional arrangement of fibers. *In vivo* experiments demonstrated that the conduit had a good guiding effect on nerve repair. Additionally, Jeffries and Wang[24] used electrospinning to fabricate multi-channel nerve conduits. *In vitro* studies show that their high surface area and porosity enhance cell penetration along the nerve-directed channels. The small-sized gaps between the electrostatically spun fibers limited the migration of most of the cells to the channel walls and minimized the influx of inflammatory cells from the surrounding tissues during implantation. The multi-channel NGC, therefore, provided an area of low growth resistance, which is more suitable for guiding axonal growth.

2.3. Porous nerve conduit

Porous structures facilitate the infiltration of cells, the diffusion of nutrients and molecular signals, and the drainage of metabolic waste products. These features promote early adhesion, spreading, proliferation, and differentiation of SCs, as well as the formation of Bungner bands and vascularization, while reducing fibrous scar formation.[47] In addition, studies have shown that the pore structure can alter the degradation rate of nerve conduits by affecting the accumulation of localized monomers and pH.[48] Particularly, Odelius et al.[49] investigated the effect of pore size on the degradability of polylactic acid (PLA) conduits and the release of degradation monomer products. Their study showed that larger pore structures can accelerate degradation through autocatalysis, whereas smaller pore sizes lead to a slower degradation rate. Wu et al.[50] investigated the *in vitro* chemical degradation properties of 3D poly(lactic-co-glycolic acid) (PLGA) porous conduits, revealing that conduits with a higher porosity or a smaller pore size degraded more slowly than the opposite conditions, thus outlasting those with a lower porosity or a larger pore size. The effects are attributed to both wall thickness and surface area size because the scaffolds with lower porosities or larger pores possess thicker pore walls and smaller surface area, which depress the diffusion of acidic degradation products, resulting in stronger acid-catalyzed hydrolysis. Song et al.[51] also investigated the degradation characteristics of porous polyester materials using a mathematical modeling strategy. The increase in porosity slows down the autocatalytic reaction and the degradation process by enhancing the diffusion coefficient of oligomers. The effect of porosity is gradually weakened as further increases in porosity lead to a reduced rate of improvement in diffusion. Numerous experimental results have also confirmed the importance of porous structures on the physicochemical and biological properties of nerve regeneration and repair, leading to their increased widespread application in the design of nerve conduits.

Nonetheless, porous nerve conduits possess certain drawbacks, including the presence of large pore sizes (normally >200 μm) that can result in fibroblast deposition, thereby hindering axonal growth. Ideal

porous nerve conduits usually have a pore size of 20–50 μm and a circular, polygonal, or longitudinal shape with a certain direction to accommodate cell permeation, nutrient exchange, and axonal guidance. However, pore morphology and distribution are usually random due to the limitations of the manufacturing methods, making it difficult to achieve a customized solution. On the contrary, 3D printing can effectively overcome these limitations, generating highly regular and consistent pore structures with a high degree of reproducibility. For instance, Tao et al.[52] developed a low-temperature gelatin porous conduit using a 3D-printed mold to promote functional recovery of transected peripheral nerves after nerve suturing. It was reported that due to its porous structure, the conduit could collapse under mechanical force and return to its original shape after absorbing saline solution. This shape memory property simplifies the conduit installation procedure, demonstrating the potential clinical application of porous NGCs in facilitating nerve sutures.

2.4. Micropatterned nerve conduit

Micropatterns on the inner surface of nerve conduits are widely used to influence cell attachment, migration, orientation, and cellular processes. One of the most used surface microstructures is the microgroove, which provides topological cues to guide cell orientation and migration in a physical model approach. Studies have shown that the *in vitro* nerve sheath can recognize the topological structure of the catheter surface[53] and extend and grow along the length of microgrooves on the plane substrate.[54]

As an example, Schmalenberg et al.[55] evaluated the ability of a microgroove-printed polymer matrix to direct the alignment of SCs. After 4 h of cultivation, more than 47% of SCs on the micro-grooved substrate were arranged within ± 20° of the groove direction, while SCs on the unpatterned substrate showed random arrangements without a clear orientation. This study found that patterned polymer matrices can enhance peripheral nerve regeneration by creating a highly ordered matrix of SCs to guide neurons. Rutkowski et al.[56] used reactive ion etching to fabricate hollow conduit lumens with microgrooves (10 μm width, 4.3 μm depth, 10 μm spacing). Such conduits combine a microfabricated matrix that guides axons at the cellular level with SCs that produce growth factors to promote regeneration. The biodegradable microgroove conduit, pre-implanted with SCs, provided physical, chemical, and biological guiding cues for axon regeneration and offered a better alternative to conventional conduits, especially for repairing sciatic nerve transactions. Davis et al.[57] prepared PLGA films with 10/10 μm and 30/30 μm microgroove structures by microlithography that sustained the release of 3 μg/cm² of FK506 for more than 56 days. In an *in vitro* evaluation of dorsal root ganglion (DRG), the released drug maintained its biological activity and significantly promoted neurite extension (average length up to the level of the positive control 10 ng/mL group). These findings suggest that this structure holds promise as a neuroprosthetic material, offering both topological guidance and sustained drug release. Yu et al.[58] utilized stamping technology to inscribe longitudinally distributed grooves and ridged surfaces of 4–5 μm depth and 5 μm width on porous PCL membranes loaded with artificial peptides. The electrophysiological recovery of the regenerated nerve was significantly enhanced in a rat model of traction sciatic nerve injury, and the compound muscle action potential amplitude was increased by more than 50% at 4 weeks postoperatively. In parallel, *in vitro* experiments showed a significant increase in both the number of SCs adhering to the membrane and their aspect ratio, validating the effectiveness of the structural–functional integration strategy for reconstructing nerve architecture and function.

2.5. Bifurcated nerve conduit

Peripheral nerves are mostly interconnected, branching structures of varying sizes, making a single tubular structure insufficient to meet the needs of complex nerve repair. On the contrary, bifurcated y-shaped nerve conduits can play an important role in inhibiting the formation of traumatic neuromas after PNI, which often leads to long-term functional deficits. Although traditional fabrication methods limit the complexity of NGC structures, AM technology has enabled the fabrication of bifurcated or y-shaped nerve conduits suitable for peripheral nerve bifurcations. Bolleboom et al.[59] 3D printed a customized y-shaped conduit and an autologous nerve graft to create a closed loop that could induce axon regeneration into the y-shaped conduit, which accurately fitted the injured proximal nerve end. This relatively simple combined approach prevented neuroma formation and significantly reduced the number of axons in the middle of the autograft, making it suitable for unilateral PNI. Zhang et al.[60] demonstrated for the first time that individual nerve stumps can form complex branching neural networks in multi-branched nerve conduits. They utilized digital light processing (DLP) 3D technology with gelatin-methacryloyl (GelMA) to construct bifurcated nerve conduits and evaluated their efficacy by transferring them from the tibial nerve to the peroneal nerve in rats. Functional and histologic evaluations showed that the bifurcation of NGC not only promoted the regeneration and functional recovery of the injured peroneal nerve but also preserved a part of the function of the donor's nerve conduit. Alternatively, Hu et al.[61] used an indirect 3D printing technique to prepare bifurcated, multi-

channel cryopolymerized GelMA gel conduits. The results suggested that such conduits could support the attachment, proliferation, and survival of adipose-derived stem cells and upregulate their neurotrophic factor mRNA expression. After implantation in rats, the bioconduits successfully supported reinnervation across a 10 mm sciatic nerve gap. The results, in both functional and histological assessments, were comparable to those of autografts, highlighting its potential clinical utility in peripheral nerve regeneration. AM can also be exploited in combination with nerve image data to successfully produce complex bifurcation nerve injuries. Johnson et al.[62] 3D-printed a rat sciatic nerve bifurcation model by scanning its anatomical structures, and the acquired data were further used to guide the preparation of bifurcated NGCs.

2.6. Selection strategies

Different structural types of nerve conduits offer distinct advantages in terms of functional performance and tissue compatibility. Therefore, selecting the most appropriate conduit type based on the specific characteristics of a nerve injury remains a critical challenge in both current research and clinical practice. A thorough understanding of the structural and functional attributes of each conduit type is essential for evaluating their suitability and developing optimal application strategies across diverse clinical scenarios, thereby enhancing the efficacy of nerve regeneration.

Hollow conduits have significant limitations in guiding directional axonal growth due to the lack of morphological cues and biochemical signals,[63] and are usually applied to short-distance nerve defects (<5 mm). In contrast, multi-channel conduits could help to minimize axonal vagrancy by providing a larger surface area for cell attachment and a more defined growth pathway, making them suitable for the repair of medium-distance (5–8 mm) defects. The porous conduit provides excellent permeability, allowing the free exchange of nutrients and molecular signals inside and outside the conduit and promoting the elimination of metabolic wastes while contributing to the adhesion, migration, and proliferation of SCs. Surface micropatterned conduits provide a significant enhancement of axonal orientation through specific microstructures, such as grooves or gradient arrangements, and are particularly suitable for the repair of larger gap defects.

In addition to the structural characteristics of the conduit, different tissue states, biological responses, and regenerative potentials at various process stages place unique requirements on the nerve conduit's selection strategy.[64] The timing of post-injury treatment can be divided into early stage (<4 weeks) and advanced stage (>4–6 weeks). In the early stage of injury, the distal nerve has not yet undergone severe degeneration. SCs still retain good proliferation and guidance ability, exhibit strong axonal growth, and show no significant local scarring.[65] At this time, excessive support is unnecessary, as a simple structural hollow conduit may be preferred to provide basic mechanical support with axonal guidance. In advanced injury, the distal nerve tissue exhibits significant degeneration and scar deposition, a decline in the number and function of SCs, a deteriorated local regenerative microenvironment, and reduced axonal regenerative potential.[66] In such cases, hollow conduits often struggle to function well, and a conduit that is more supportive, such as multi-channel conduits, porous structure conduits, or micropattern conduits, should be chosen. In addition, in advanced nerve injury, scar tissue formation is one of the key factors hindering regeneration. Massive deposition of collagen with other extracellular matrices leads to tissue sclerosis, which forms a physical barrier, interferes with the direction of axonal growth, and may induce the formation of neuromas. Therefore, the selection of nerve conduits with anti-scarring or pro-regenerative functions is crucial in advanced nerve repair. Micropatterned conduits can effectively guide the directional growth of axons and reduce the obstruction of regeneration by scarring. The characteristics of different conduit structures and their suitability are summarized in Table 2. In summary, the selection of appropriate NGC structures not only facilitates the reconstruction of axonal guidance pathways but also optimizes cellular behavior and promotes the localized accumulation of neurotrophic factors, thereby accelerating nerve regeneration and enhancing functional recovery.

3. Biomaterials for nerve conduit

The structure of the conduit significantly influences the overall outcome of the nerve regeneration process. Nevertheless, structural design alone is insufficient to achieve optimal nerve repair; the choice of materials also plays a critical role. Properties of the conduit material, such as biocompatibility, mechanical performance, degradation rate, and bioactivity, directly impact its functionality during nerve regeneration.[67] Therefore, a synergistic interplay between material and structure is essential. A proper design of the structure provides the necessary space and orientation for nerve repair, while suitable materials ensure that the structure remains stable in the physiological environment to support the growth, proliferation, and differentiation of neural cells. The organic integration of both elements is key to achieving effective nerve regeneration.

The selection of biomaterials for NGCs is based on the following requirements: (i) good processability, (ii)

Table 2. Nerve guidance conduit structures and their advantages and disadvantages

Structure	Structural characteristic	Advantages	Disadvantages	Application
Hollow nerve conduit	Single hollow, no internal filling	Simple structure, easy to manufacture, facilitates the migration of various cells and the accumulation of neurotrophic growth factors	Lack of morphological and biochemical cues; limited regeneration efficiency	Shorter nerve defects, typically less than 5 mm; early damage
Multi-channel nerve conduit	Multiple longitudinal channels, simulated nerve bundle	Provides a larger surface for cell attachment, facilitates migration of Schwann cells, and reduces axonal dispersion	Complex structure, difficult to manufacture, and susceptible to permeability and degradation	Medium length nerve defects, 5–8 mm; advanced injuries
Porous nerve conduit	Porosity in the conduit wall	Allows infiltration of cells, nutrients, and molecular signals, as well as excretion of metabolic wastes	Excessive pore size leads to deposition of fibroblasts and hinders axonal growth; pore distribution is often uneven	Medium length nerve defects, 5–8 mm; advanced injuries
Micropatterned nerve conduit	Microstructure with microgrooves, ridges, etc., on the inner surface	Promotes cell-directed migration and axonal alignment	High manufacturing precision; difficulty in fabricating	5–10 mm; potential to repair longer nerve defects and advanced injuries
Bifurcated nerve conduit	Y-shaped or multi-branch structure	Suitable for complex nerve branch repair; prevents neuroma formation	Difficult to manufacture; requires personalized design	Bifurcated and multi-path nerves

good biocompatibility and bioactivity, and (iii) suitable physicochemical properties, such as mechanical strength and structural stability. The materials for 3D-printed NGCs can be categorized into natural or synthetic polymers based on the type of embedded cells. These biomaterials and representative NGC examples (Table 3) are discussed further.

3.1. Natural polymers

Natural polymers are macromolecular compounds present in living organisms and have re-emerged in the last few decades as major bioactive substances due to the presence of biofunctionalized and bioactive molecules with biomimetic properties and natural recombination. Most natural materials are biocompatible and can be rapidly degraded *in vivo*,[68–70] promoting cell adhesion, migration, growth, and proliferation while avoiding the toxic effects caused by synthetic materials.[71] Excellent biocompatibility, minimal immunogenicity, and the ability to support cell growth make them excellent candidates for the fabrication of NGCs. Chitosan, silk fibroin (SF), gelatin, and collagen are natural polymers commonly used for the 3D printing of nerve conduits.

3.1.1. Chitosan

Chitosan (and derivatives) is a biodegradable linear polysaccharide, obtained from shrimp shells or crustaceans, with excellent biocompatibility and antimicrobial properties. The presence of groups such as amines/aminos and hydroxides on its molecular chain allows cells to better adhere and grow on its surface. In addition, chitosan has good processability and easy degradation in animals.[72] It has been used as an NGC material by controlling its degradation time without the need for secondary surgical removal. Pure chitosan has a tensile modulus of about 20–50 MPa and a modulus of elasticity of 0.5–1.5 GPa.[73] Despite the many advantages, its low strength, the absence of temperature sensitivity, and its shear-thinning behavior limit its applications in the field of biofabrication. Therefore, chitosan is often used in combination with other materials to enhance its mechanical properties and provide a more stable conduit structure. By crosslinking chitosan with high-performance synthetic polymers such as PLA or PCL, the mechanical properties can be significantly enhanced. The resulting composites typically have a tensile strength of 100–150 MPa and a modulus of elasticity of more than 3–4 GPa, making them more suitable for application in scenarios that require high mechanical properties, such as nerve conduits and bone tissue engineering.[73] For instance, Nawrotek et al.[74] prepared chitosan/PCL conduits doped with bioactive agent microspheres using electrodeposition combined with extrusion printing. The structural properties of the conduits did not change significantly during incubation at 37°C in phosphate buffer solution (pH 7.4) for up to 28 days, demonstrating good structural stability. Bianchini et al.[75] prepared porous 3D-printed chitosan/PCL using genipin cross-linking to improve the conduits' physicochemical properties. The results demonstrated that compared to chitosan conduits, the hydration rate of the genipin-cross-linked conduits was significantly reduced, with the equilibrium constant of 470.3 ± 29.7%, which is much smaller than that of the chitosan conduits (642.3

Table 3. Biomaterials and representative examples of nerve guidance conduits

Materials	Method	Conduit structure	*In vitro*/*in vivo*	Model	Culture time	Resolution	Outcomes	References
Chitosan/neurotrophic factor-3	Topography method	Hollow	*In vivo*	15 mm remote nerve defects in rats	12 weeks	10 μm	Effective functional recovery in a rat model of 15 mm sciatic PNI	[76]
Chitosan/PCL	3D melt extrusion	Spiral hollow conduit	*In vitro*	*In vitro* assessment of cell behavior	28 days	1 mm	The structural properties did not change significantly after incubation in phosphate buffer solution, showing good structural stability	[77]
Chitosan/poly-ε-caprolactone	Extrusion-based 3D printing and freeze-drying	Porous	*In vitro*	*In vitro* assessment of cell behavior	3 months	–	Genipin incorporation significantly reduced the degradation rate of the catheter matrix and stabilized the chitosan matrix	[78]
Chitosan/PCL	Extrusion printing	Microstructure modification	*In vivo*	15 mm remote nerve defects in rats	4 months	1 mm	The printed patterns showed excellent flexibility and toughness, capable of withstanding continuous compressive and bending stresses	[164]
Silk fibroin	Electrospinning	–	*In vivo*	10 mm nerve defects in rats	12 weeks	–	Low-molecular filipin composite conduit with good biosafety and degradability; promotion of sciatic nerve regeneration in rats	[83]
PPy/SF	3D electrospinning	–	*In vitro*	*In vitro* assessment of cell behavior	30 days	–	SCs mainly adhered to the surface of the conduits and maintained a normal morphology on the PPy/SF conduit	[84]
SF/dorsal root ganglion/SCs	Injection molding	Hollow	*In vivo*	10 mm nerve defects in rats	12 weeks	–	Enhancement for PNI's repair by incorporating neural equivalents into SF-based conduits	[157]
SF	Electrospinning	Multi-channel		–	–	1 mm	Bionic multi-channel functionalized nerve guidance conduits with comparable mechanical behavior to that of the rat sciatic nerve	[80]
Antheraea pernyi SF/poly(L-lactic acid-co-caprolactone)/graphene oxide nanofibers	Electrospinning	Multi-channel	*In vivo*	10 mm sciatic nerve defect in rats	12 weeks	10 μm	3D biomimetic nerve scaffolds had the ability to offer an effective guiding interface for neuronal cell growth	[173]
PPy/SF	Electrospinning	Double-deck	*In vivo*	10 mm sciatic nerve defect in rats	6 months	0.1 mm	Longitudinally guided PPy/SF electrically conductive composite conduit with good performance for clinical applications	[81]
Collagen/hydrogel/poly(l-lactic acid-co-ε-caprolactone	Electrospinning	–	*In vivo*	8 mm nerve defects in rats	12 weeks	–	Aligned collagen hydrogels are favorable for nerve regeneration as a directional guidance pathway	[85]
Collagen/hyaluronic acid/PCL	Electrospinning	–	*In vitro*	*In vitro* assessment of cell behavior and neuronal differentiation	21 days	–	3D composite conduit with a permissive environment for the proliferation and maturation of SCs, allowing axonal growth	[86]

(*Continued...*)

doi: 10.36922/IJB025140120

Table 3. Continued...

Materials	Method	Conduit structure	*In vitro*/*in vivo*	Model	Culture time	Resolution	Outcomes	References
GelMA/Engelbreth–Holm–Swarm	3D printing	Hollow	*In vitro*	*In vitro* assessment of cell behavior	16 weeks	0.1 mm	Confirming that the Engelbreth–Holm–Swarm hydrogel filling accelerates the repair of nerve defects	[92]
GelMA/methoxy PEG-PCL	Digital light processing	Hollow	*In vivo*	10 mm nerve defects in rats	3 months	–	Gelatin conduit as a physical microenvironment for axonal extension and the promotion of functional recovery after PNI	[93]
GelMA/bone marrow stem cells/PEGDA	Additive lathe 3D bioprinting	Double-deck	*In vitro*	PC12 cell culture *in vitro*	21 days	1 mm	Enhancement of conduit's mechanical properties by adding a biocompatible GelMA/PEGDA hydrogel layer	[94]
Poly(lactic acid)/poly(ε-caprolactone)/graphene	Extrusion printing	porous	*In vitro*	*In vitro* assessment of cell behavior	7 days	300 μm	The stent has a modulus of elasticity of approximately 22.36 MPa and is suitable for peripheral nerve tissue applications	[100]
PLA	Extrusion printing	Hollow	*In vivo*	3 mm nerve defects in rats	56 days	0.1 mm	PLA support for axonal growth as a potential alternative to autografts	[101]
PLA/PCL	Stereolithography	Microgroove	*In vitro*	*In vitro* assessment of cell behavior	7 days	10 μm	PCL/PLA films have higher Young's modulus and cell proliferation trends than PCL films	[102]
PCL/collagen nanofibers/graphene oxide	Electrospinning	Hollow	*In vivo*	10 mm nerve defects in rats	8 weeks	10 μm	Improvement of the mechanical properties of conduits by the addition of graphene oxide doping in PCL	[172]
PCL/decellularized extracellular matrix/PDA	Fused filament fabrication	Hollow	*In vitro*	*In vitro* assessment of cell behavior and neuronal differentiation	7 days	100 μm	Higher mechanical properties of PCL conduit than sciatic nerve; easy surgical manipulation and suturing	[107]
Collagen I/PLGA	Electrospinning	Hollow	*In vivo*	13 mm nerve defects in rats	12 weeks	0.1 mm	Collagen/PLGA conduit for SC proliferation and alignment on nanofiber conduits compared to random orientation	[111]
Poly(l-lactic acid-co-ε-caprolactone/PLGA/PPy	Electrospinning	–	*In vitro*	*In vitro* assessment of cell behavior and neuronal differentiation	7 days	10 μm	High rate of biodegradation for PLGA-based conduits; more suitable for short-distance nerve defects	[112]
Polyurethane/PEG-graphene oxide	Stereolithography	Hollow, porous, and micro-grooved		–	–	0.1 mm	The addition of PEG's functional groups helps to improve the mechanical strength of the conduit	[144]
PEG	Stereolithography	Multi-channel		–	–	0.01 mm	Rigid PEG-fibrinogen constructs have been shown to be better at promoting complete bridging at the injury site compared to fibronectin/PEGDA	[114]

Abbreviations: 3D, three-dimensional; GelMA, gelatin methacryloyl; PCL, polycaprolactone; PEG, polyethylene glycol; PEGDA, poly(ethylene glycol) diacrylate; PLA, polylactic acid; PLGA, poly lactic-co-glycolic acid; PNI, peripheral nerve injury; PPy, polypyrrole; SC, Schwann cell; SF, silk fibroin.

doi: 10.36922/IJB025140120

± 31.6%; $p < 0.01$). The degradation rate of the conduit was significantly reduced, and the structural stability was enhanced. Zhang et al.[76] utilized chitosan/neurotrophic factor-3 for the 3D-printed topology micron track conduit to induce targeted growth of SCs, showing a higher growth density of primary SCs on micron track conduit compared to commercial conduits, which effectively facilitated the functional recovery of a 15 mm gap nerve injury model in rats.

3.1.2. Silk fibroin

SF is obtained from silkworm silk, which is a rich natural source of proteins with outstanding mechanical properties, biodegradability, biocompatibility, and bioabsorbability.[77–79] Silk can be processed in the form of films, gels, nets, etc., having a wide range of applications in NTE, especially using electrospinning. For instance, both Dinis et al.[80] and Zhao et al.[81] electrospun SF-based 3D NGCs. SF-NGCs had an ultimate peak stress of 4.0±0.6 MPa and a corresponding elongation at failure of 156.8±46.7%, demonstrating that the SF-NGCs exhibited a mechanical behavior comparable to that of rat sciatic nerve, good clinical application performance, and potential to promote nerve regeneration and functional recovery. Wang et al.[82] combined electrostatic spinning, braiding, and coating techniques to prepare a composite sericin protein NGC, which was implanted into a 10 mm nerve defect in rats. There was no obvious inflammatory reaction after 8 weeks of implantation. Apparent axonal and myelin tissue appeared inside the conduit group at 12 weeks and directly throughout the conduit tissue, indicating successful regeneration of the rat sciatic nerve within the conduit. Alternatively, Zhao et al.[83] prepared composite polypyrrole (PPy)/SF conduits cultured with SCs by combining 3D bioprinting with electrospinning. The results showed that SCs mainly adhered to the surface of the conduits and maintained normal morphology (full, pike-shaped, and shiny), good interconnection, and good proliferation. This indicated that the composite PPy/SF conduit promoted SC adhesion, differentiation, and proliferation. However, obtaining SF is a complex and expensive process that demands specialized methods and resources. Moreover, variations in silk sources and processing parameters can affect the material's quality and consistency.

3.1.3. Collagen

Collagen is the most abundant protein in mammals, with good biocompatibility, high stability, and the capability to promote axonal regeneration and myelination. Collagen types I and II are the key components of peripheral nerves. Several studies explored collagen and its derivatives as the main material for the biofabrication of NGC. For instance, Fujimaki et al.[84] developed a collagen-based NGC with a guiding effect and bridged a 15 mm peripheral nerve defect in rats; myelinated nerve regeneration was observed at 8 weeks postoperatively, showing good nerve repair. Yoo et al.[85] successfully 3D-printed a natural, unmodified collagen nerve conduit using a dense collagen solution and implanted it into a rat nerve model. 6 weeks after implantation, the 3D-printed NGC showed a denser and more organized pattern of regenerating axons. Morphometric analysis of nerve sections distal to the repair site at 12 weeks showed that myelinated axon counts and myelin thickness were higher in the 3D-printed group than in the control group, confirming the beneficial effects of 3D-printed collagen on axonal regeneration, myelin sheath regeneration, and nerve cells' functional recovery. However, the main drawbacks of collagen are its high cost, poor mechanical properties, and limited stability, as it can only remain liquid at low temperatures. To address this limitation, cross-linking or combining collagen with synthetic materials is often necessary. For instance, Chen et al.[86] fabricated porous 3D-printed collagen/SF (C/S) conduits adsorbed with secretome (ST) derived from human umbilical mesenchymal stem cells (3D-C/S+ST). After co-culture with NSC, 3D-C/S+ST showed good cytocompatibility, and infrared spectroscopy data showed that 3D-C/S+ST had appropriate lipid-soluble and water-soluble chemical bonds suitable for neuronal cell adhesion and growth. From X-ray diffraction analysis, 3D-C/S+ST also showed a desirable crystallinity, hence better control of the rate of degradation. Therefore, the development of collagen-based composites for NGC fabrication is a common procedure to be considered.

3.1.4. Gelatin methacrylate

Gelatin is a natural polymer derived from collagen[87] and has excellent biocompatibility, hemostatic properties, low cytotoxicity, and antigenicity, and promotes cell attachment and growth.[88] However, its mechanical properties and antimicrobial activity are relatively poor, with a tensile strength that is generally 0.05–0.5 MPa.[89,90] Therefore, it often needs to be crosslinked with other polymers to promote its mechanical performance.[91] GelMA is a gelatin-based biomaterial chemically modified to promote cell adhesion, growth, and degradation. GelMA has better mechanical tunability and structural stability than ordinary gelatin. Its mechanical strength and degradation rate are dependent on many factors, such as gelatin concentration, degree of methacrylation, and photo-crosslinking.

Gong et al.[92] proposed a hollow porous GelMA/Engelbreth–Holm–Swarm (EHS) NGC with 89.8% porosity, 167.6 μm pore diameter, 0.489 kPa tensile modulus, and 0.314 kPa compressive modulus. 16 weeks after implantation in rats, hematoxylin and eosin staining

of the gastrocnemius muscle showed that there was no significant difference between the autograft group and the composite conduit group, confirming that the EHS filling accelerated the repair of nerve defects. Tao et al.[93] 3D printed hollow GelMA hydrogels with drug-loaded poly(ethylene glycol)-poly(3-caprolactone) nanoparticles to obtain a physical microenvironment for axon extension and drug release for nerve regeneration. *In vitro* experiments showed that the migration of SCs increased with the drug concentration. The conduit was placed into rats, and the functional recovery of injured peripheral nerves was detected through electrophysiology. The results showed that the efficacy of the 3D-printed conduits was comparable to that of autografts, having a good clinical application value. Liu et al.[94] also repaired a bilayer NGC using GelMA/bone marrow mesenchymal stem cells (BMSCs)/gelatin, which resulted in a high survival rate and extensive morphological expansion of BMSCs encapsulated in the inner layer. The density and value-added rate of PC12 cells attached to the cellularized bilayer NGCs were four and nine times higher than those of the non-cellularized bilayer NGCs, respectively. These results suggested that the 3D bioprinting of BMSCs embedded in bilayer NGCs has great potential to promote peripheral nerve repair. Another example is reported by Ye et al.,[155] which exploited photocurable GelMA to fabricate multi-channel NGCs using DLP technology. Neural crest stem cells cultured on the GelMA NGC showed good survival, proliferation, and migration rates.

In summary, although most natural polymers exhibit low cytotoxicity and possess unique biological advantages, such as the antibacterial activity of chitosan and the neuroregenerative potential of collagen, they still face several challenges in practical applications. Some of these challenges include poor mechanical strength, uncontrollable degradation rates, and complex processing and purification procedures. To overcome these limitations, current research should focus on modifying natural polymers through chemical or physical approaches to enhance mechanical properties and achieve more controllable degradation profiles while preserving inherent biocompatibility and bioactivity. This could help in improving the applicability of natural polymers in nerve conduits and other areas of regenerative medicine.

3.2. Synthetic polymers

Synthetic polymers are artificial polymers with adjustable chemical structures and physical properties, categorized into degradable and non-degradable materials. Degradable materials have been widely used due to their material's inherent plasticity, wide range of sources, lower cost, good biocompatibility, and controllable physicochemical properties.[95] Commonly used biodegradable synthetic polymers include PLA, PCL, PLGA, and polyethylene glycol (PEG). Non-degradable materials are instead less commonly used due to induced chronic foreign body reaction, which inhibits the recovery of peripheral nerve function and requires a secondary surgical procedure to remove them.[4]

3.2.1. Polylactic acid

PLA is a biodegradable thermoplastic aliphatic polyester derived from plants and contains repeating lactic acid units that promote the proliferation, migration, and maturation of myelinated axons.[96,97] The Young's modulus (around 4.1 GPa) and tensile strength (around 62.7 MPa) of PLA are significantly higher than most synthetic conduit materials, implying that PLA-based NGCs can effectively maintain morphology and support nerve regeneration in complex *in vivo* environments. Its degradation product is lactic acid, which can be rapidly metabolized by the body, but its poor cell adhesion and high hydrophobicity limit its application.[98,99] Gerdefaramarzi et al.[100] fabricated PLA/PCL/graphene nanocomposite conduits for peripheral NTE using a fused filament fabrication 3D printing method, which showed porosity and pore sizes in the range of 50– 86% and 300–500 μm, respectively. The elastic modulus of the scaffolds was approximately 22.36 MPa, which is suitable for peripheral nerve tissue applications, and the degradation rate in phosphate buffer solution was 0.14 mm/day, which is very close to the regeneration rate of peripheral nerve tissue. These findings suggest that PLA/PCL/graphene oxide conduits are promising for peripheral NTE. Wang et al.[101] also prepared a composite NGC using chitosan/PLA and used it for bridging long segments of peripheral nerve deficits. The results showed that 6 months after grafting, innervation was restored to the target muscles and limb mobility was improved. Alternatively, Yue et al.[102] prepared film-based surfaces with micro-grooved NGCs on PCL/PLA using micro-stereolithography (SLA). Young's modulus of PCL/PLA films (~109.6 MPa) was higher than that of PCL films (~56.7 MPa), which indicated that the stiffness and deformation resistance of PCL/PLA were improved. PCL/PLA samples showed a higher human neuroblastoma cell proliferation than PCL and longer cellular lengths (~275 μm), which was higher than that of control PCL films (~200 μm). Hence, this facile fabrication method is promising for the fabrication of morphology-guided cues. Zheng et al.[103] developed a combinatorial NGC consisting of longitudinally aligned electrospun nanofibers and porcine decellularized nerve matrix hydrogel. The *in vivo* capacity for facilitating nerve tissue regeneration and functional recovery was evaluated in a rat sciatic nerve defect model. PLA-aligned/0.25% porcine decellularized nerve matrix hydrogel scaffold exhibited the

best performance in facilitating directed axonal extension and SC migration *in vitro* due to the combined effects of the topological cues provided by the aligned nanofibers and the biochemical cues retained in the porcine decellularized nerve matrix hydrogel. Consistent results were obtained in animal experiments with the fabricated NGCs, confirming that PLA is generally a promising approach for NTE and the treatment and diagnosis of PNI.

3.2.2. Polycaprolactone

PCL is a biodegradable aliphatic polyester characterized by a low melting point, ease of processing, high mechanical strength, good biodegradability, and miscibility with various other polymers.[104] However, due to its high plasticity, the physical, chemical, mechanical, and biological properties of PCL can be improved by modifying functional groups or combining it with other materials (e.g., synthetic polymers, metallic materials, etc.).[105] PCL-based NGCs support the adhesion and proliferation of SCs and olfactory sheath cells without causing severe swelling that may locally compress the nerve. Zhu et al.[106] employed electrospinning techniques to fabricate a PCL-based NGC featuring directionally aligned fibers complemented by a concentration-gradient NGF coating on its surface. This innovative design facilitated the directional guidance of DRG neurons toward the high NGF-concentrated region and successfully repaired a 15 mm sciatic nerve defect in rats. Chen et al.[107] tested the mechanical properties of 3D-printed PCL conduits with maximum tensile strength and modulus of elasticity of 21.2 and 142.5 MPa, respectively—values significantly higher than those of native nerves. This enhanced strength offers greater ease of handling and suturing during surgical procedures. Qian et al.[108] innovatively invented an LBL casting method for the fabrication of NGCs, utilizing either single-layer graphene or multi-layer graphene with PCL. Results showed that the higher the number of layers of graphene, the lower the conductivity achieved (8.92×10^{-3} S/cm for a single-layer graphene/PCL conduit, 6.37×10^{-3} S/cm for a multi-layer graphene/PCL conduit). All the above-mentioned studies demonstrate that NGCs made from PCL have great potential for future research and clinical applications. In addition to this, even with synthetic polymers, hybridization with other materials is still an essential way to enhance the performance of conduits.

3.2.3. Poly(lactic-co-glycolic acid)

PLGA is a functional polymeric organic compound formed by the random polymerization of two monomers, lactic acid and hydroxyacetic acid, with a controlled biodegradation rate[109] and good cell adhesion. Although the degradation rate of PLGA reduces with increasing lactic acid content, it remains significantly higher than that of PLA and PCL.[110] Ouyang et al.[111] developed a seamless axial NGC consisting of biocomposite collagen/PLGA nanofibers by electrospinning and evaluated the ability of the conduit to support SC proliferation and axonal growth both *in vitro* and *in vivo*. The aligned NSCs were significantly superior to those composed of randomly aligned collagen/PLGA fibers in facilitating regeneration and functional restoration and were nearly as effective as autologous grafts. Moreover, the collagen/PLGA conduits were mechanically strong enough to suture severed nerve endings and resist collapse *in vivo*, remaining structurally intact for at least 3 months postoperatively. Namhongsa et al.[112] deposited polypyridine particles on 3D-printed poly(l-lactic acid-co-ε-caprolactone)/PLGA conduits to change the electrical conductivity of the conduit surface. Studies have shown that PLGA-based conduits degrade faster and are more suitable for short-gap nerve injuries, and vice versa. By optimizing the structural and surface modifications of the conduits, these materials can provide a favorable option for enhancing cellular biocompatibility. The controlled degradation properties of PLGA are also a valuable factor for achieving multi-phase release of neurotrophic factors. For instance, Lackington et al.[113] developed a PLGA microparticle with encapsulated NGF and glial-derived neurotrophic factor. The factors are sequentially released into the nerve regeneration microenvironment over 28 days, promoting nerve repair. However, PLGA generally exhibits poor hydrophilicity, and its degradation products create an acidic microenvironment, often resulting in limited bioactivity.

3.2.4. Polyethylene glycol

PEG is a linear polyether compound with strong mechanical and degradation properties that can encapsulate cells, good hydrophilicity, and water absorption. However, due to its biological inertness and poor oxidative stability, it may cause cell death during ultraviolet cross-linking. Therefore, it is typically combined with other biomaterials to improve its performance.

Berkovitch et al.[114] sought to evaluate hydrogel assemblies PEG-fibrinogen (Fib) in different formulations for NGCs. Three PEG-Fib hydrogels were prepared and tested: compliant PEG-Fib, rigid PEG-Fib, and micropatterned PEG-Fib with microchannels. All three compositions were implanted into 8 and 12 mm rat sciatic nerve defect models to verify their effectiveness in repairing PNI. Rigid PEG-Fib constructs containing 40 mg/mL of PEG diacrylate (PEGDA) were shown to be better at promoting complete bridging at the injury site compared to fibronectin/PEGDA. The semi-synthetic compositions were superior to fibronectin and fibronectin/PE-DA in bridging nerve injuries. In addition, PEG conduits promoted homeostasis

in vivo by providing cells with an adhesion and degradation substrate. By employing SLA, Farzan et al.[144] used solvent-free polyurethane (PU) and different contents of PEG-graphene oxides (0, 0.5, 1, 3, and 5 wt%) to prepare composites. Among all samples, the composites containing 5 wt% PEG-graphene oxide exhibited the highest tensile stress (3.51±0.54 MPa), tensile rupture strain (~170%), and compressive strength, suggesting that the addition of PEG's functional groups improved the mechanical strength of the conduit.[116] Similarly, Arcaute et al.[142] used SLA-printed multi-lumen PEG-based NGCs, demonstrating that high PEG concentrations, especially from 20 to 30 wt%, were more resistant to suture pullout, with a significant increase in resistance from 0.043±0.0037 to 0.064±0.0090 N/mm.

To summarize, it has been observed that the current choice of conduit materials is shifting toward the utilization of hybrid polymers. By adjusting the composition and mixing ratio of these composite materials, it is possible to achieve a controllable regulation of the biodegradation rate and mechanical strength. These adjustments may be able to meet the different needs of specific types of injuries and injury gaps to promote better PNI repair and regeneration.

3.3. Cells and biomolecules

Assisted by certain advanced technologies, living cell lines and biomolecules can now be directly used as raw materials for 3D printing.[117] The integration of living cells with biomaterials to form bioinks not only enhances the biocompatibility of the printed constructs but also effectively reduces immune rejection and inflammatory responses. In particular, the incorporation of cells can provide structural support derived from the native ECM, thereby promoting the adhesion, growth, and survival of neural cells and ultimately improving the efficiency of nerve regeneration. Moreover, specific biomolecules, such as neurotrophic factors and growth factors, can be co-loaded with cells into the conduit structure, where they play a synergistic role in regulating cellular behavior and enhancing the efficacy of nerve repair during the regeneration process.

3.3.1. Stem cells

Stem cells for NTE mainly include embryonic stem cells, induced pluripotent stem cells, NSCs, and mesenchymal stem cells (MSCs) of different tissue sources.[118] The induction efficiency of NSCs greatly varies due to the heterogeneity of species, age, tissues from which they are derived, and culture conditions.[119] Thus, many studies have focused on the use of MSCs.[120,121] The use of undifferentiated MSCs for *in vivo* studies allows pluripotent cells to differentiate MSCs along multiple pathways by advancing axons and natural SC stimulation. This helps to create a conducive environment for nerve regeneration.[122] In addition, transplanted MSCs have shown the ability to differentiate into other support cells, such as endothelium-like cells, smooth muscle cells, or pericytes.[123] These endothelium-like cells can produce various growth factors, such as vascular endothelial growth factor (VEGF), which have been shown to have synchronous effects on angiogenesis, neurogenesis, and nerve regeneration, with positive effects on *in vivo* nerve regeneration.[124]

Zhang et al.[125] isolated a unique subpopulation of MSCs from human gingival tissue and found that this cell type tends to be induced into neural progenitor cell-like cells. In a rat model of facial segmental defect, gingival MSC spheres were printed as the sole cellular component using 3D bioprinting. Results indicated that axon regeneration and target muscle recovery reached the same level as autogenous nerve transplantation at 12 weeks after implantation. Cui et al.[126] attempted to bridge the 3.5 cm dog sciatic nerve defect with a longitudinally oriented collagen catheter (LOCC) loaded with human umbilical cord mesenchymal stem cells (hUC-MSCs). 9 months after the operation, the connective tissue of the nerve stump at the injured site was collected for tissue chemical analysis. Compared with the control group, the positive signal in the middle ganglia of the LOCC/hUC-MSCs group was more intense. Transmission electron microscopy analysis of the thickness and size of the regenerated myelin fibers showed that the LOCC/hUC-MSC group had a thicker myelin sheath on the nerve fibers regenerated at the midpoint of the ganglia than the LOCC group.

3.3.2. Schwann cells

SCs are non-neuronal cells but provide support and protection to neurons by forming myelin sheaths, constituting supportive factors for maintaining homeostasis within the peripheral nervous system. In PNI, SCs serve as the primary glial cells promoting axonal regeneration.[127] Due to rapid phenotypic changes, they form favorable growth pathways and ultimately generate myelin phospholipids around axons in response to PNI. Therefore, incorporating SCs in conduits is highly encouraged for peripheral nerve repair, and controlling their orientation through 3D bioprinting is expected to effectively guide the directed growth of neurites.

Ning et al.[128] developed a bioprinting process to prepare SC-coated conduits using a novel hydrogel mixture. The observed hydrogel microstructure and cell morphology revealed that the coated SCs exhibited superior performance in environments with high concentrations of fibronectin, as it could provide sufficient micropores and fibers for cell metabolism and adhesion. Although low concentrations of fibronectin could provide cell-binding fibers within the hydrogel, insufficient porosity would

still constrain SC diffusion and phenotype expression. Therefore, an appropriate porosity is a necessary condition for the successful preparation of NGCs encapsulating SCs. Furthermore, Ning et al.[129] researched bioprinting SC-loaded conduits using low-viscosity hydrogel components such as RGD-modified alginate, hyaluronic acid, and fibronectin. Experimental results indicated that bioprinting could induce the alignment of SCs. By controlling the printing speed, the elongation of SCs could be effectively regulated, thereby adjusting the alignment of DRG neurites. As the speed increased from 4 to 9 mm/s, the roundness of SCs increased, and the direction of laminin expression became more apparent. Alternatively, in the study of Summa et al.,[130] SCs were implanted into hollow fibronectin conduits and implanted into a 10 mm rat sciatic nerve model. Compared to control hollow conduits, conduits filled with differentiated BMSCs, or conduits filled with differentiated adipose-derived stem cells, these fibronectin-SC conduits exhibited significantly enhanced nerve regeneration capabilities. All these evidences demonstrate the effective role played by SCs in nerve regeneration.

3.4. Biomolecules

In the process of peripheral nerve repair, various types of biomolecules, especially neurotrophic factors, play an indispensable role in promoting axon guidance, cell migration, SC activation, and nerve regeneration. Integrating these biomolecules into NGCs not only helps to create a microenvironment conducive to regeneration but also serves as a key strategy to enhance the biological activity of the conduits, providing a more precise and efficient intervention for the treatment of nerve injury.

The NGF is one of the earliest neurotrophic factors discovered and mainly promotes the growth of sensory and sympathetic nerves. *In vitro* experiments have demonstrated that NGF promotes the survival, proliferation, and synapse growth of sensory and sympathetic neurons.[131] Histological and morphological studies have shown that NGF treatment significantly increases the number and diameter of myelinated nerve fibers and accelerates the recovery of electrophysiological parameters after sciatic nerve damage.[132] In a 14 mm rat sciatic nerve defect model, researchers utilized NGF gradient immobilization within a nanofiber conduit to significantly guide the directional growth of DRG axons and enhance the recovery of nerve morphology and function, with results comparable to those of autologous nerve grafts.[133] In addition to NGF, other commonly used neurotrophic factors mainly include brain-derived neurotrophic factor, VEGF, and fibroblast growth factor 2 (FGF2). Brain-derived neurotrophic factor has a critical role in the repair of both the central and peripheral nervous systems, especially in the later stages of nerve regeneration, and can significantly promote axon growth and neural network remodeling.[134] VEGF provides the basis for the restoration of nutrient supply to the injured area, mainly by promoting neovascularization. *In vitro* experiments have shown that VEGF promotes the proliferation of SCs by activating signaling pathways. Related *in vivo* studies have shown that the number of regenerated nerve fibers in the VEGF-treated group is almost double that of the hollow conduit control group.[135] FGF2, also known as the basic fibroblast growth factor, is considered to be the most important molecule in promoting nerve regeneration among the 23 members of the fibroblast growth factor family.[136] It has been found that the use of collagen conduits co-modified with FGF2 and ciliary neurotrophic factor significantly improved nerve electrophysiological function and tissue remodeling in a model of long segmental defects in the nerve.[137]

In summary, the integration of neurotrophic factors into nerve conduits provides an important strategy for constructing a functional nerve repair microenvironment. By optimizing the type, dosage, release profile, and spatial distribution of these factors within the conduit structure, it is expected to achieve precise spatial-temporal regulation of the nerve regeneration process, which can significantly enhance the regenerative quality and clinical effects of PNI treatment.

4. Three-dimensional bioprinting technologies for nerve conduits

A significant aspect of NGC research is the optimization of technological approaches to achieve high precision and superior performance in NGC fabrication. In recent years, the application of 3D printing to tissue engineering has revolutionized NGC manufacturing. Among the various NGC biofabrication techniques, 3D bioprinting has been extensively investigated. This method is often combined with 3D imaging tools to develop personalized conduits with micro- or nano-scaled anatomical accuracy tailored to the patient's injury site. In particular, 3D bioprinting allows the assembly of both biological and non-biological elements in a 3D organization to produce bioengineered structures for regenerative medicine, pharmacokinetics, and cell biology research. This technology facilitates the positioning and localization of cells and biomolecules within functionalized biomaterials into inks to replicate the complex nerve ECM and accurately mimic the 3D structure of neural tissue fibers. Consequently, 3D bioprinting can integrate a wide range of components into a single printed structure, making it a powerful tool in nerve regeneration research.

3D bioprinting technologies are divided into three main categories: light-based, extrusion-based, and jet-based printing. Light-based technologies, such as SLA and DLP, are usually not used for bioprinting due to the cytotoxicity of photo-initiators present in the inks. Extrusion-based printing can serve as a 3D bioprinting technique when bioink is incorporated. Other advanced technologies, such as the recently developed Kenzan method, enable bioprinting by constructing fully biological NGCs directly from cells, eliminating the need for biopolymer solutions or hydrogels. The technical principles and main materials utilized for the various technologies are presented in Figure 5, while Table 4 summarizes the advantages and disadvantages of selected 3D bioprinting techniques.

4.1. 3D printed nerve guiding conduit

SLA is an AM technique that constructs 3D components by using a laser beam to scan and cure a photopolymer resin surface in an LBL process. As one of the rapidly developing 3D printing technologies in biomedical engineering, SLA has the advantages of high resolution, smooth surface,[138] and the ability to fabricate complex structures.[139] Although SLA can achieve resolutions up to 30 μm, it is a relatively time-consuming and discontinuous process, which requires additional post-processing steps to fully cure the printed part to obtain mechanically strong and stable building blocks.[140] The main process parameters are laser power, print speed, print layer thickness, and light time. Unlike other applications, SLA in medical contexts mostly uses visible lasers rather than ultraviolet light, as visible wavelengths are less likely to cause cytoplasmic and genomic destruction of printed cells.[141] Several case studies have exploited SLA for the biofabrication of NGC, verifying their feasibility in guiding the regeneration of nerve injuries. Arcaute et al.[142] used SLA to print a multi-channel PEG neural conduit. They found that varying the printing speed and laser intensity impacted the mechanical strength and dimensional accuracy of the conduit. A higher laser speed (~20.5 cm/s) resulted in insufficient mechanical strength post-crosslinking, causing the conduit to deform easily. Gels with good mechanical strength can be crosslinked when using lower laser speeds (3.5 cm/s) (Figure 6A-iii). Conversely, slower laser speeds improved mechanical strength but led to a loss of dimensional accuracy (Figure 6A-iv). An energy level of 65 mJ/cm^2 (laser scanning speed of 10.5 cm/s) was found to crosslink a good pattern with high geometrical precision and adequate strength (Figure 6A-ii). Evangelista et al.[143] also used SLA to test the promotion of single-channel (Figure 6B-i) and multi-channel conduits (Figure 6B-ii) for PNI repair using PEG as the material. It was found that the number of regenerating axons in single-channel NGCs approached that of normal nerves, and that multi-channel conduits showed significantly less nerve regeneration, possibly due to the physical nature of the conduit that inhibits growth. Further studies are needed to evaluate the role of the multi-channel NGCs. Farzan et al.[144] synthesized biodegradable, electrically conductive, solvent-free PU/PEG-graphene oxide composites and successfully printed them into neural conduits with different precise geometries, such as hollow, porous, and microgroove conduits (Figure 6C). It was found that the composites exhibited the highest tensile stress (3.51±0.54 MPa), strain at break (~170%), and electrical conductivity (1.1 × 10^{-3} S/cm) when the graphene oxide content was set at 5 wt%. In addition, 5 wt% PU/PEG-graphene oxide had higher compressive strength

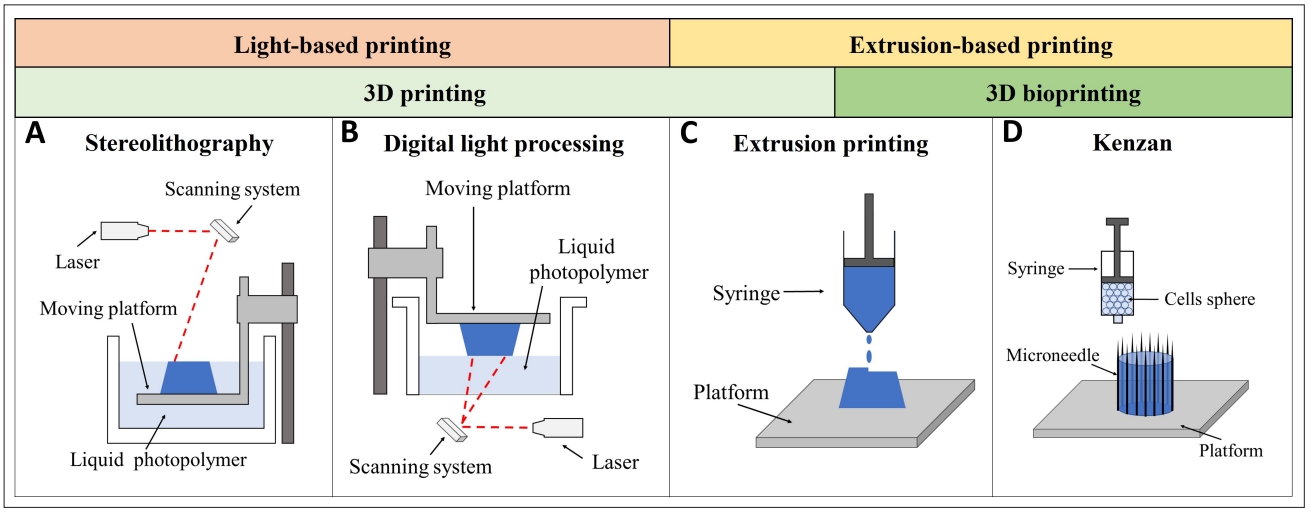

Figure 5. Three-dimensional (3D) bioprinting methods. (A) Stereolithography, (B) Digital light processing, (C) Extrusion printing, and (D) Kenzan.

Table 4. Advantages and disadvantages of three-dimensional bioprinting technologies

Three-dimensional bioprinting technology	Advantages	Disadvantages
Stereolithography	High resolution (~30 μm); ability to manufacture complex structures	Requirement of additional post-processing steps to cure the printed part, a time-consuming printing process, and high material waste; photoinitiators may be toxic
Digital light processing	High resolutions (~25 μm); simultaneous cross-linking within layers for fast production speeds; low viscosity materials; fast light curing capability	Limited selection of photopolymerizable biomaterial resins, photoinitiators may be toxic, and the high price of the printer
Extrusion printing	Suitable for a wide range of materials; printability of bioinks at high viscosity and cell density; low cost and relatively simple printing process	Reduction in cell viability after extrusion; low resolution down to 200 μm
Kenzan	Fully functional biological nerve guidance conduits can be constructed without the need for biopolymer solutions or hydrogels, relying only on cells	Conduit size is vulnerable to change during fusion of cell spheres, with low dimensional accuracy

compared to pure PU and exhibited proper enzymatic degradation after 6 weeks, which is expected to last long enough for effective nerve regeneration. Perez et al.[145] modified a commercial SLA printer with a sterile system to produce clean, particle-free conduits and reduced the laser diameter to increase its micromachining capability for the fabrication of complex conduit structures. Singh et al.[146] prepared hollow, multi-channel, and factor-filled biodegradable NGCs using SLA (Figure 6D), which could bridge a 15 mm nerve injury gap in rats. It was shown that the synthesized NGC was biocompatible and could provide guidance for the migration and proliferation of nerve cells, which improved the rate of nerve regeneration.

4.2. Digital light processing

Similar to SLA technology, DLP uses digital light projection to polymerize or cure the entire photoactive resin for each layer,[147] which in turn enables the layered fabrication of 3D structures. The main advantage of DLP over SLA is the rapid production of 3D structures. The laser source in SLA systems crosslinks the resin at each laser spot,[148] whereas the introduction of digital micromirror devices in DLP promotes the rapid crosslinking of the entire layer. Similar to SLA, c, such as a limited selection of photopolymerizable biomaterial resins, strong odors caused by the polymerization between acrylate groups and photoinitiators, and higher resin waste, which increases the cost of printed parts.[149–151] For DLP, the resolution of the projector determines the accuracy of the print, which is currently up to 25 μm,[152] allowing more detailed microstructures of NGCs to be printed. Numerous scholars have used DLP to conduct experiments on rational NGC printing, and NGCs fabricated using DLP have demonstrated their ability to support peripheral nerve regeneration. Xu et al.[153] established a DLP 3D bioprinting system to fabricate NGCs with oriented and continuous microstructures. The researchers fabricated NGCs with different inner diameters, wall thicknesses (Figure 7A-i), and microfibers (Figure 7A-ii), and tested the effect of functionalized nerve conduits loaded with HDAC3-specific inhibitor (RGFP966) nanoparticles after implantation into 10 mm nerve defects in rats. The results showed that the nerve conduction velocity of the loaded group was 29.83 m/s, which was significantly higher than that of the unloaded group (23.49 m/s), demonstrating the promoting effect of RGFP966 on functional recovery. Tao et al.[154] used DLP to fabricate natural polymers and platelets into NGCs. The compression test showed that the conduit had good mechanical properties and could recover the printed structure after deformation with structural integrity to support peripheral nerve regeneration. In addition, enzymatic degradation studies have shown that the conduit could be degraded *in vitro*, eliminating the requirement of a secondary surgical resection after implantation. As a light-curing technology, exposure time is an important process parameter in the printing process. Ye et al.[155] explored the optimal exposure time for GelMA in the DLP preparation of multi-channel neural conduits. To avoid the potentially harmful effects of ultraviolet light, a 405 nm visible light source was used to cure the biomaterial ink. During printing, the size of the conduit was affected by the layer exposure time, light intensity, and layer thickness. Printability was analyzed by adjusting the layer exposure time while other printing parameters remained constant. Conduits printed with shorter exposure times (<20 s) (Figure 7B-i) exhibited poor mechanical properties and were easily deformed. In contrast, longer exposure times enhanced the mechanical strength (Figure 7B-iii), but resulted in significant blockage at the base of the multi-

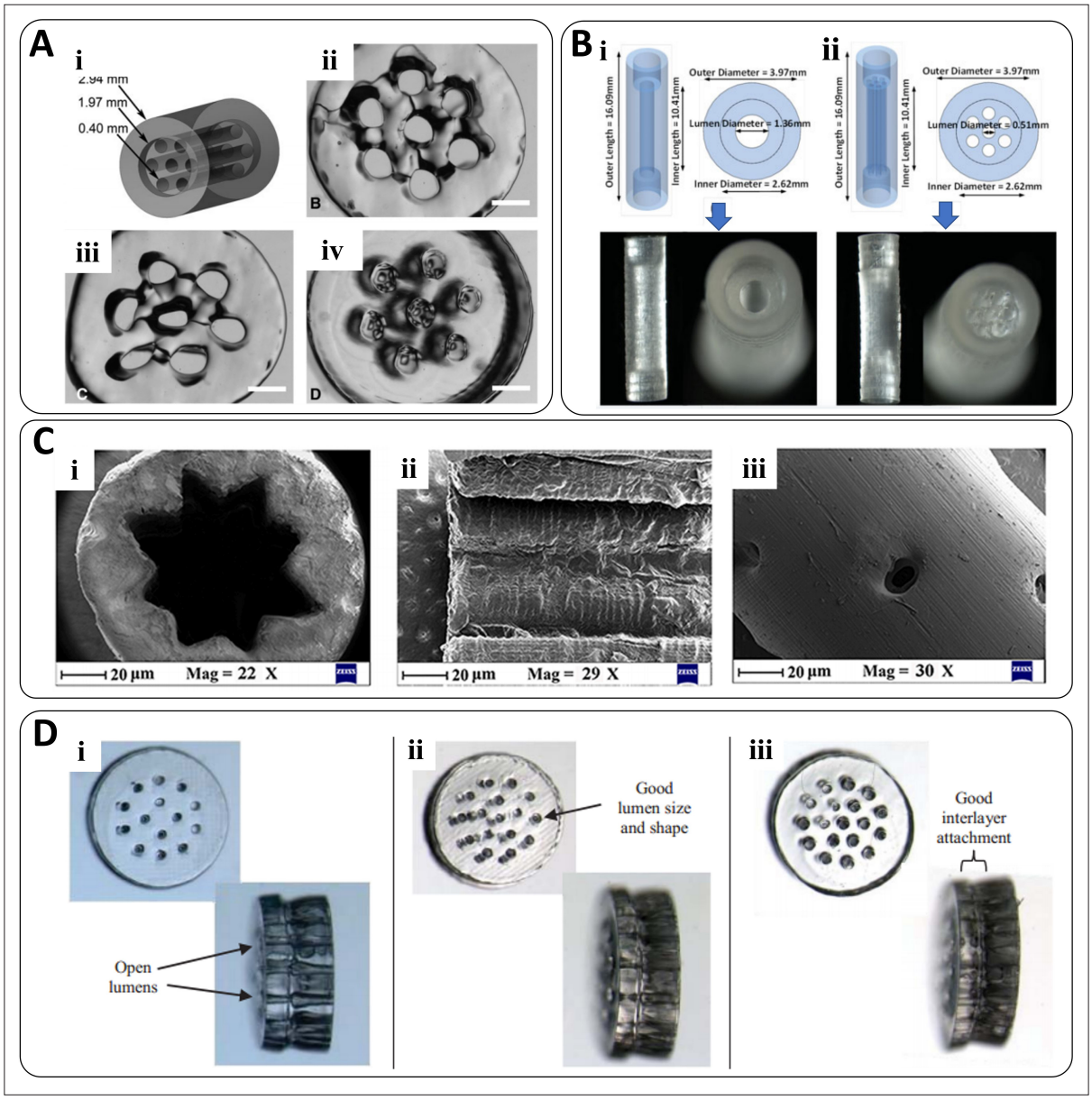

Figure 6. Various conduits prepared using SLA technology. (A) Polyethylene glycol (PEG) nerve guidance conduits. (i) Three-dimensional (3D) design model crosslinked at (ii) appropriate laser scanning speed or energy, (iii) high laser scanning speed or low energy, and (iv) low laser scanning speed or high energy. Scale bar: 500 mm. Reprinted with permission from Ref.[142] Copyright© 2011 Mary Ann Liebert Inc. (B) (i) Single-channel and (ii) multi-channel conduit design dimensions with pre-implantation pictures. Reprinted with permission from Ref.[143] Copyright© 2015 Thieme Medical Publishers. (C) Scanning electron microscopic (SEM) images of (i) transverse section and (ii) longitudinal section of grooved 3D-printed conduits. (iii) SEM images of stereolithography and porous 3D-printed conduit prepared using 5% polyurethane/PEG-graphene oxide. Scale bar: 20 µm, magnifications: 22×, 29×, and 30×. Image adapted from Farzan et al.[144] (D) SEM micrographs of (i) hollow, (ii) 4-pore, and (iii) open pores conduits. Scale bar: 200 µm. Reprinted with permission from Ref.[146] Copyright© 2018 American Chemical Society.

channel structure due to over-curing. An exposure time of 35 s fulfilled the requirements for manufacturing an NGC (Figure 7B-ii) with good mechanical strength and the required dimensions, at a more appropriate exposure time (which is not a generalized duration, but provides a data reference for other experiments). Huang et al.[156] successfully DL-printed NGCs with aligned structures from poly (glycerol sebacate) acrylate (Figure 7C-i) and poly (glycerol sebacate) acrylate-polyvinylpyrrolidone. The aligned microgroove structures could be observed from the NGCs' tomographic view (Figure 7C-iii). The microgroove structure effectively guided the growth

direction of nerve cells, promoting cell proliferation during nerve tissue regeneration. Wu et al.[157] used DLP to prepare an active hydrogel composed of GelMA and SF methacrylate, which had a positive effect on the adhesion, proliferation, and migration of SCs. A 12 mm sciatic nerve defect model was established in rats, and a conduit was implanted. Electrophysiological, morphological, and histological evaluations demonstrated that the conduit could promote axonal regeneration, myelin sheath regeneration, and functional restoration by providing a favorable microenvironment, with strong potential for the treatment of long-gap peripheral nerve injuries.

4.3. Extrusion printing

Extrusion printing is the most widely used 3D printing technique in NTE. The material is loaded and continuously extruded from the print head through a nozzle and selectively deposited LBL according to a pre-generated path,[158,159] with an acceptable resolution between 200 and 2000 μm. Extrusion printing allows the deposition of biomaterials with relatively high viscosity and shear-thinning effect, which withstands during the printing process, preventing any collapse. Therefore, porous conduits with excellent structural integrity, such as neural conduits, can be fabricated. Extrusion 3D printing offers high deposition through (multi) nozzles at high printing speeds and low equipment costs. However, the system is prone to nozzle clogging and pressure drops, which may eventually lead to cell apoptosis.[160,161]

Vijayavenkataraman et al.[162] developed an extrusion-based 3D printing machine to fabricate NGCs using a conductive collagen/PPy-block-PCL hydrogel. The authors investigated the effect of printing speed and material flow rate on the printed path width (Figure 8A), demonstrating that, as the print speed increases, the material is subjected to tension, and the path width narrows or is discontinued. At higher material flow rates, the ink may also aggregate into irregular shapes rather than a continuous line, affecting the structure of the NGC. Although low-viscosity materials are often a good choice for maintaining cell viability during and after printing, high-viscosity materials can provide better support and fidelity during the printing process. Therefore, the right trade-off between printing speed and flow rate was demonstrated to be critical. Kaplan et al.[163] also printed poly(L-lactic acid)/PLGA conduits

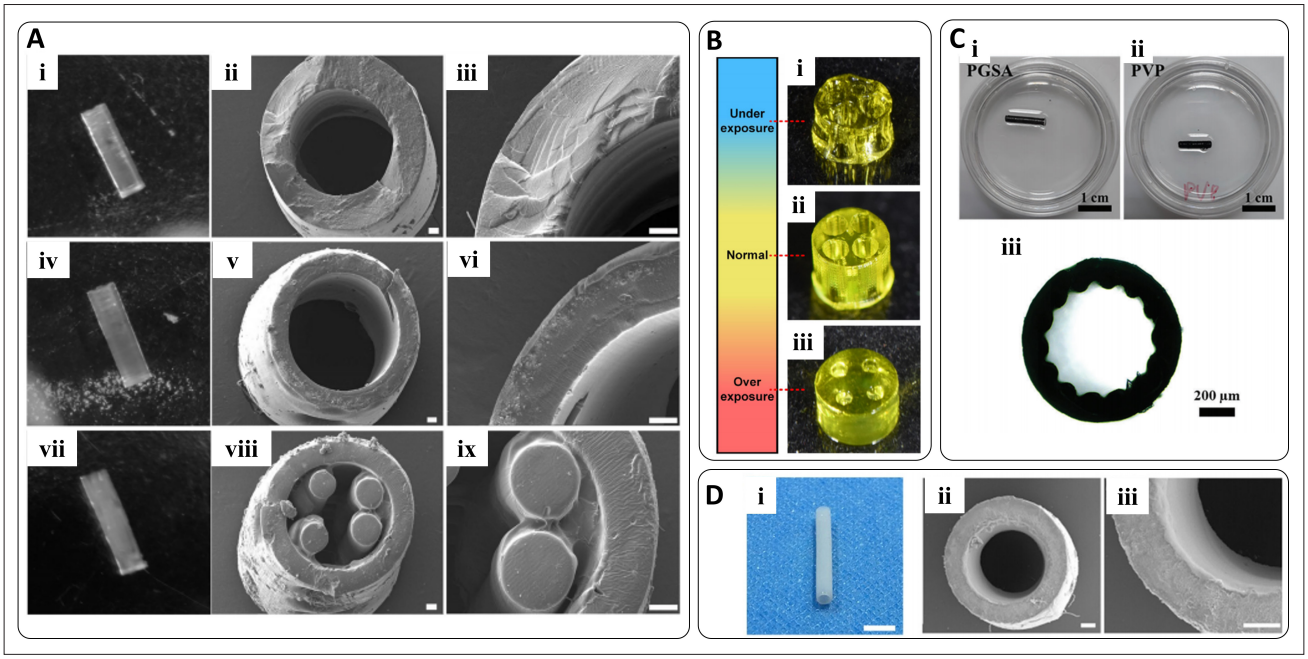

Figure 7. Various conduits prepared using DLP technology. (A) Hydrogel nerve guidance conduits of different structural designs. (i) Thick-walled, (ii) thin-walled, and (iii) microfibers printed using digital light processing technology. Scale bar: 100 μm. Reprinted with permission from Ref.[153] Copyright© 2019 Elsevier. (B) Multi-channel patterned photo crosslinked images at different exposure times. (i) Insufficient exposure time (<20 s), (ii) appropriate exposure time (around 15 s), and (iii) very long exposure time (>50 s). Reprinted with permission from Ref.[155] Copyright© 2020 Elsevier Ltd. (C) Poly(glycerol sebacate) acrylate (PGSA) composite micro-grooved conduits. (i & ii) Images of three-dimensional-printed PGSA and PGSA-polyvinylpyrrolidone (PVP) conduits. (ii) Optical microscope images of the cross-section of PGSA composite micro-grooved conduits. Scale bars: 1 cm and 200 μm. Reprinted with permission from Ref.[156] Copyright© 2023 Wiley. (D) (i) Photographs of the conduit and (ii & iii) scanning electron microscopic images of the conduit. Scale bar: 5 mm. (ii & iii) Scale bar: 200 μm. Reprinted with permission from Ref.[157] Copyright© 2015 Elsevier.

(Figure 8B) with random pore orientations. Stromal cells were implanted onto the fiber-conjugated protein-coated conduits, and after 7 days, the cells had populated the microchannels and exhibited a diffuse morphology, suggesting that the conduits had adequate cell culture and attachment conditions. Redolfi-Riva et al.[164] designed and prepared honeycomb mesh PCLs of different sizes using extrusion printing and embedded them in a chitosan porous matrix (Figure 8C). The hexagonal structural units provided enough space to ensure nutrient exchange and impede the infiltration of fibrotic tissues while constituting the structure of the conduit. As a result, the printed patterns showed excellent flexibility and toughness, capable of withstanding continuous compressive and bending stresses. Histological analysis showed that all rats treated with these grafts had abundant cellularization in the walls and lumens of the tubes as well as in the regenerated axons. Englanda et al.[165] also successfully fabricated fibronectin factor XIII-hyaluronic acid hydrogel conduits (Figure 8D) containing SCs using extrusion bioprinting.

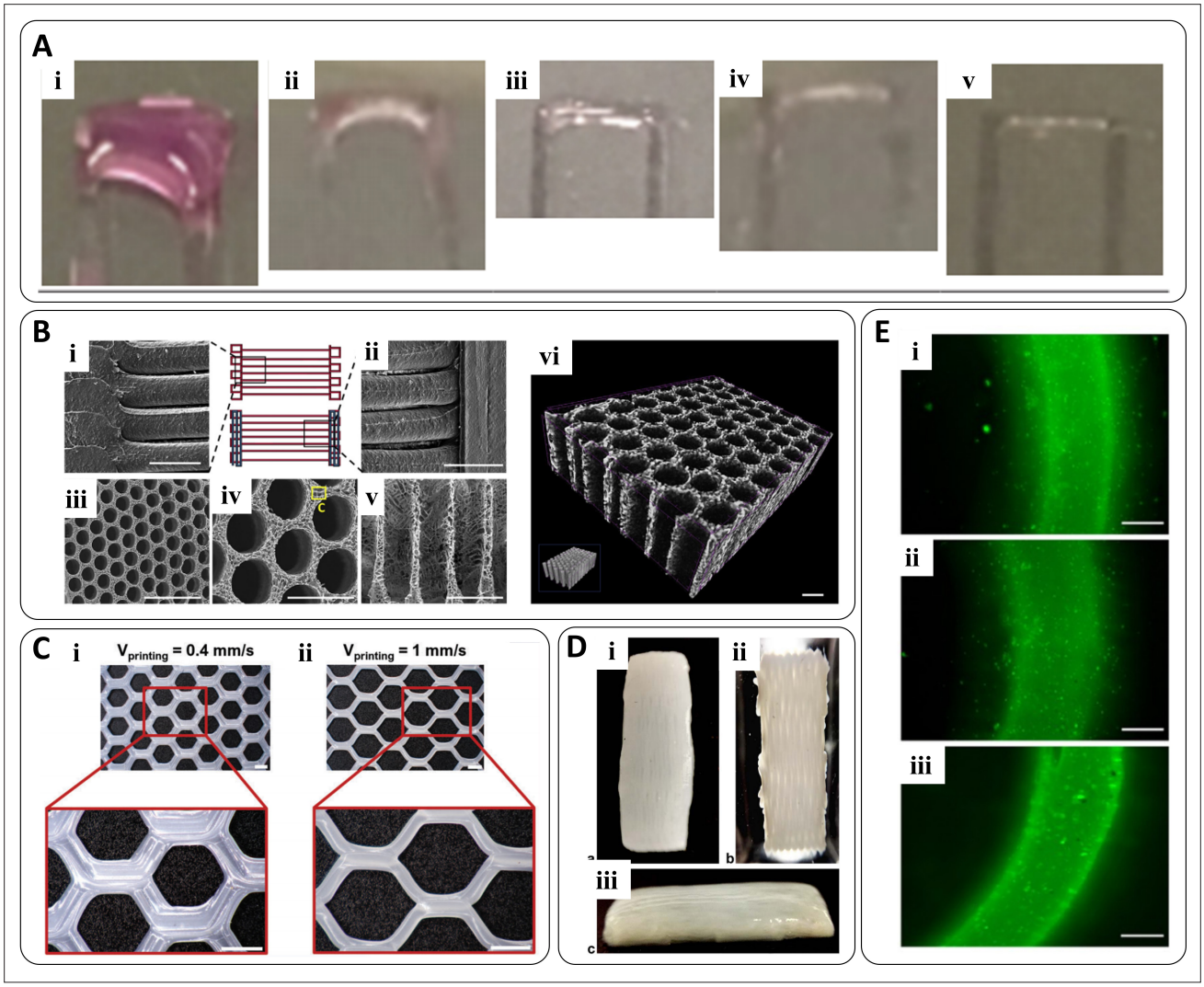

Figure 8. Various conduits prepared using the extrusion printing technique. (A) Effect of printing speed (i) 1 mm/s, (ii) 3 mm/s, (iii) 5 mm/s, (iv) 7 mm/s, and (v) 10 mm/s on fiber angle for printing collagen at a flow rate of 3 mL/min. Reprinted with permission from Vijayavenkataraman et al.[162] Copyright© 2019. (B) (i) Scanning electron microscopic images. (i–v) Scanning electron microscopic images. (i & ii) Scale bar: 200 μm. (iii) Scale bar: 1 mm. (iv) Scale bar: 400 μm. (v) Scale bar: 500 μm. (vi) Computed tomography diagram of poly(L-lactic acid)/PLGA conduit. Scale bar: 200 μm. (ii) Computed tomography diagram of poly(L-lactic acid)/PLGA conduit. Scale bar: 200 μm. Reprinted with permission from Ref.[163] Copyright© 2022 Elsevier Ltd. (C) Conduits at two different printing speeds (i) 0.4 mm/s, (ii)1 mm/s. Scale bar: 1 mm. Image adapted from Redolfi-Riva et al.[164] (D) (i) Frontal, (ii) dorsal, (iii) lateral views of fibrin-factor XIII-hyaluronic acid conduits. Reprinted with permission from Ref.[165] Copyright© 2017 Elsevier BV. Scaffold dimensions: 14 × 5 × 2 mm. (E) LIVE/DEAD staining of N2a-laden three-dimensional bioprinted 7.5G, 7.5G0.1C, and 7.5G7.5P. Images were taken 5 days after bioprinting. The green color represents healthy cells, and the red represents dead cells. Scale bar: 200 μm Reprinted with permission from Ref.[166] Copyright© 2024 American Chemical Society.

During the bioprinting process, SCs were encapsulated within the conduit, promoting the longitudinal alignment of fine fibrin fibers. The physical guidance cues provided by these longitudinally aligned fibrin fibers further directed the SCs to align linearly and facilitated linear neurite elongation along the fibrin factor XIII-hyaluronic acid chain. Finally, Das et al.[166] formulated cell-carrying biobricks with Neuro-2a cell density of 3×10^6 cells/mL into GelMA/carbon nanofiber/PEGDA/gellan gum hydrogels and printed them into two layers of cylindrical conduits (wall thickness of 0.5 mm) using a customized extrusion bioprinter. After 5 days of incubation in a differentiation medium, the Neuro-2a cells showed good cell viability (more than 80%) (Figure 8E).

4.4. Kenzan

The Kenzan method uses pre-designed 3D data and stainless steel microneedle arrays as temporary scaffolds on which multi-cellular spheres are precisely placed. Once these spheroids fuse and produce their own ECM, the microneedles are removed, leaving behind the desired biological structure. This method does not require a biopolymer solution or hydrogel, relying solely on cells to construct fully biological conduits with a certain degree of biomechanical stability. This approach pioneers a new direction in NTE.[167] Needle diameters are typically 100–200 μm with a needle pitch of 300–400 μm. The size of the multi-cellular spheres is determined by the needle spacing. The distribution of cells within the sphere undergoes a continuous rearrangement, with cells exhibiting stronger adhesion migrating toward the core, while those with weaker adhesive properties localize to outer concentric layers in decreasing order of adhesion strength. Inside the sphere, cells move within the available space and limits imposed by intercellular adhesions. This process, combined with ECM deposition, promotes the healing of pinholes. However, since the goal is to print tubes or hollow structures, this contraction may cause them to disappear prematurely, which would require additional stabilization.

Yurie et al.[168] used the Kenzan method to prepare a conduit-free hollow NGC (Figure 9A) from human normal dermal fibroblasts and verified its restorative effect on sciatic nerve defects in rats. Human dermal cells were first aggregated to form homogeneous multi-cellular spheres with a diameter of 750 ± 50 μm, and the spheres were arranged into a 3D shape according to a pre-designed 3D model (Figure 9A-i). After about a week of printing, adjacent spheres were fused together to construct a single tubular shape in a microneedle array, which was then removed (Figure 9A-ii). *In vivo* experiments demonstrated that 3D-bioprinted fibroblast conduits showed a significantly higher number of myelinated axons (Figure 9A-iii) compared to the silicone group (Figure 9A-iv), which could promote nerve regeneration in a rat sciatic nerve defect model. Although there are some limitations, the study confirmed that this new purely biological NGC is effective in promoting nerve regeneration. Alternatively, Takeuchi et al.[169] explored an approach for larger-gap nerve defect repair. A 12 mm NGC (Figure 9B-i & ii) was prepared using the Kenzan method and implanted into a 10 mm sciatic nerve defect in the right hind limbs of rats. A silicone tube implantation group was used as a control. Evaluation was performed 8 weeks after surgery, in the distal region of the suture site, where the number of myelinated axons, myelin sheath diameter, and myelin sheath thickness of regenerated axons were significantly greater in the NGC (Figure 9B-iii) than in the silicone (Figure 9B-iv) groups. It was eventually shown that the NGCs could promote peripheral nerve regeneration, even in a 10 mm nerve defect model. On this basis, Yurie et al.[170] further investigated the promotion of nerve regeneration using NGCs generated from bone marrow stromal cells. NGCs were fabricated with the Kenzan method and transplanted into Lewis rats to bridge the 5 mm right sciatic nerve gap (Figure 9C-i), with two silicone tubes used as control (Figure 9C-ii & iii). Functional and morphological evaluation of nerve regeneration was performed 12 weeks after transplantation. Electrophysiological studies, kinematic analysis, wet muscle weight, and morphological parameters showed that nerve regeneration in the NGC was significantly better than in the silicone tube. Mitsuzawa et al.[171] further investigated the effect of this method on nerve defect regeneration promotion in large mammals. Spheres were extracted from a 96-well plate into a fine nozzle, strung into an array of circular needles (Figure 9D-i & ii), and developed into a tubular structure according to a predesigned pattern (Figure 9D-iii). An NGC (Figure 9D-iv–vi) was used to bridge the ulnar nerve defect in a dog's 5 mm forelimb, and nerve regeneration was observed at 10 weeks postoperatively. Immunohistochemical, histologic, and morphometric assays confirmed the presence of numerous myelinated axons in the NGC. It was shown that the NGC fabricated from canine autologous dermal fibroblasts promoted nerve regeneration even in a 10 mm nerve defect model. This technique is therefore feasible for the preclinical treatment of PNI and segmental nerve defects.

5. Clinical translation and commercial application: moving toward scalable solutions

This review reports the high potential of biofabricating NGCs for nerve tissue repair, focusing on their structure, AM-oriented technology, biomaterials, and cells. The

reported NGC prototypes have all been extensively tested in specific case studies, which validated their efficient use. Nevertheless, their exploitation and clinical translation remain limited. Several key challenges must be addressed before NGCs can be widely adopted in preclinical and clinical studies. These challenges are mainly related to the selection of appropriate biomaterials and technologies, as highlighted in the previous sections. For biomaterials, European Medicines Agency or Food and Drug Administration approval is a critical requirement. Several biomaterials have already been approved for a variety of medical applications, including PLA, PLGA, and collagen. For instance, NeuraGen® and NeuroMatrix® are Food and Drug Administration-approved nerve conduits made from collagen type I and primarily used for small-diameter peripheral nerves with short defects. Particularly, NeuraGen® is a tube filled with chondroitin-6-sulfate, which promotes SC migration and axonal regeneration. Their functionality was demonstrated in rat sciatic nerve gap models to increase nerve fiber density and myelinated axon counts, and was comparable to reversed autografts and superior to hollow or collagen-filled conduits.

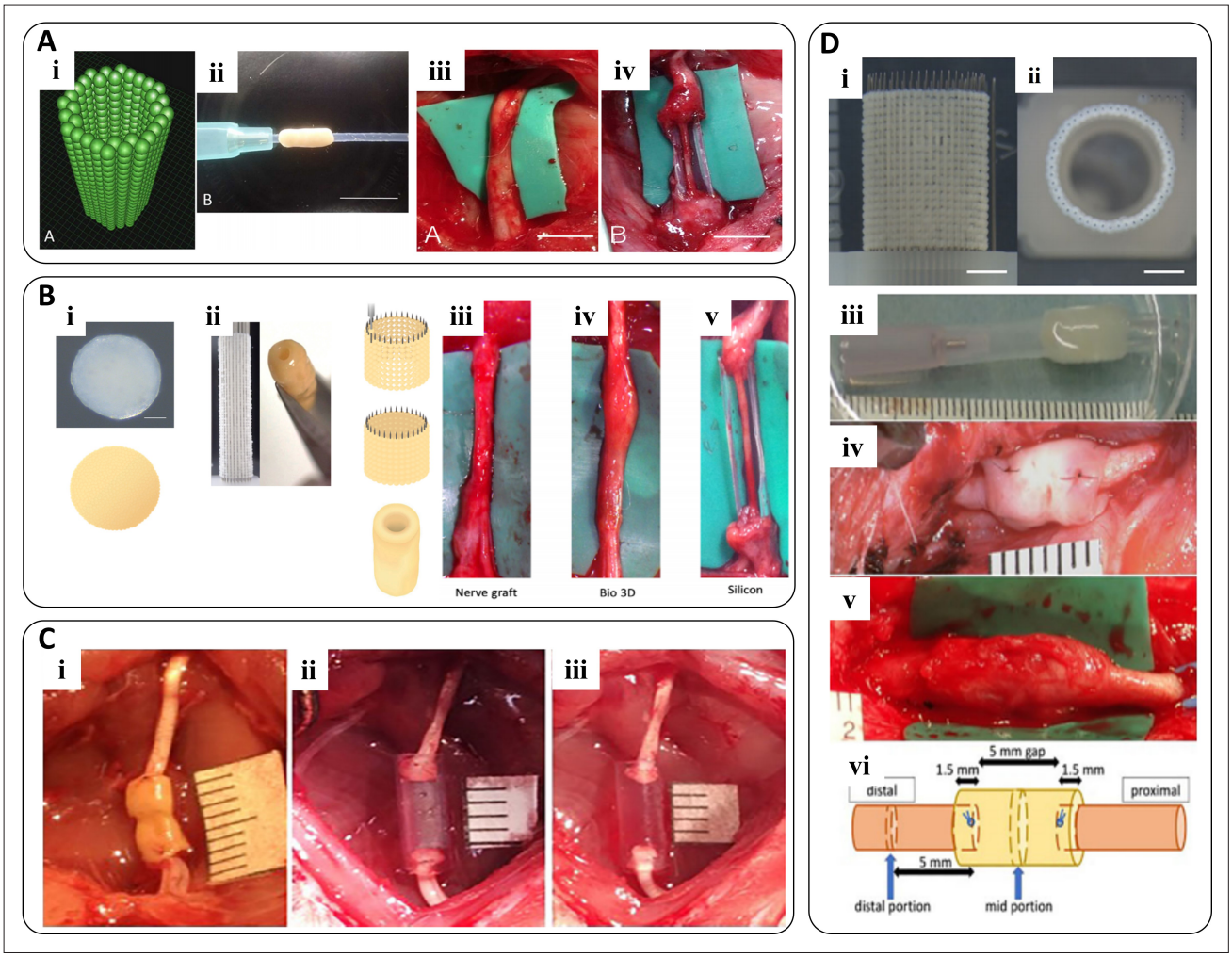

Figure 9. Various conduits prepared using Kenzan technology (A) (i) Pre-designed three-dimensional (3D) tubular structure (green spheres represent homogeneous multi-cellular spheres, cultured only from normal human dermal fibroblasts). (ii) Bio-3D conduit and (iii) bio-3D group regenerates the sciatic nerve 8 weeks after surgery. (iv) Regeneration of the sciatic nerve in the silicone group at 8 weeks postoperatively. Scale bar: (ii) 10 mm. (iii & iv) 5 mm. Adapted from Yurie et al.[168] (B) (i) Fibroblasts aggregate to form spheroids and (ii) fabrication of bio-3D nerve guidance conduits with three types of conduits bridging the 5 mm gap of rat sciatic nerve: (iii) nerve graft group, (iv) bio-3D group, (v) silicon cell group (SC group). Reprinted with permission from Ref.[169] Copyright© 2020 Wiley. (C) (i) Bio-3D group, (ii) silicone group (S group), (iii) (silicone cell group) SC group. Reprinted with permission from Ref.[170] Copyright© 2020 Cognizant Communication Corporation. (D) Stringing of the multi-cellular spheres into circular needle arrays according to a pre-designed pattern (i) from the side, (ii) from above, and (iii) the bio-3D conduit before transplantation. (iv) Insertion of 8 mm bio-3D conduit into nerve defect. (v) Ulnar nerve regeneration at 10 weeks postoperatively. (vi) Schematic of resection and graft dimensions. Scale bar: (i & ii) 2mm Reprinted with permission from Ref.[171] Copyright© 2019 Sage Publications.

However, despite their innovative vision and current implementation in clinical studies, these conduits are still limited to small-diameter nerves and may not be effective for bridging longer nerve gaps. Advancements in biomaterials and 3D bioprinting technologies[115] can therefore offer the potential for developing novel nerve conduits that address such limitations by incorporating bioactive factors to further enhance nerve regeneration, while offering more complex NGC geometrical structures. The use of composite biomaterials functionalized with biomolecules or embedded with SCs or NSCs also inevitably complicates the certification process. The regulatory approval typically focuses on certifying the core biomaterial, such as the scaffold, while the integration of patient-derived cells is subject to separate approval processes. Consequently, one of the biggest obstacles at the moment is regulatory approval. Furthermore, most of the research to date has been based on small animal models, which do not fully capture the complexity of human peripheral nerve regeneration. Validation using large animal models is necessary to accurately mimic human anatomical and physiological conditions. Ultimately, the scalability of such prototypes heavily depends on factors like batch-to-batch variation, long-term sterility, scalability, and rigorous quality control, all of which are crucial for successful product commercialization. For customized or multi-material structures, the scalability of bioprinting platforms remains a significant challenge. Advancements in process automation, closed-loop control systems, and real-time quality assurance technologies will increase their technological availability and market awareness. Within this framework, stronger cooperation between all stakeholders involved, including academic researchers, physicians, regulatory agencies, and business partners, is needed to move forward.

6. Conclusion

NGCs have a broad application prospect in the field of nerve repair for their ability to construct NTE conduits using a combination of designs and biomaterials chosen to mimic the natural structure of peripheral nerves and provide a favorable microenvironment for nerve regeneration. Although there are various current solutions, most of these conduits are still in the experimental stage and have not reached clinical translation. None of them can currently fully substitute autologous nerve grafts to repair peripheral nerve defects. Recently, AM has been exploited for the fabrication of multi-material and multi-functional NGCs. An ideal 3D-bioprinted personalized NGC should mimic the precise structural details of the nerve region to be replaced by guiding tissue regeneration. In this concept, biocomposites of natural and synthetic origins are seen as a promising approach to achieve controlled regulation of the biodegradation rate, mechanical strength, and other properties of the conduit. This customization allows the conduit to meet the specific requirements of different types of injuries and injury gaps, thereby providing superior performance in the repair and regeneration of PNIs.

Although 3D bioprinting has made significant progress in the fabrication of peripheral NGCs, and the prospects are bright, many challenges remain. The biomaterials available for printing are limited, and the performance in terms of degradability, biocompatibility, and mechanical properties needs to be improved. Future research on NGCs should focus more on enhancing their bio-hybridity by controlling the embedment of stem cells with neurotrophic factors and targeted drugs. This strategy aims to further expand the potential for nerve regeneration, addressing the various needs of nerve repair effectively. In addition, future studies of nerve repair mechanisms will provide a better direction for the design of new 3D-printed NGCs. The integration of bioelectronic interfaces, smart biomaterials, and 4D printing is another emerging direction in the field of NGCs that has the potential to completely transform the next generation of NGCs. The development of NGCs that adjust to the physiological conditions of the regenerating nerve is achievable through 4D printing, which involves the generation of dynamic structures capable of responding to environmental stimuli, such as temperature, pH, or electrical signals. The regenerative capacity of NGCs could also be enhanced using smart biomaterials engineered to actively release bioactive molecules or exhibit a controlled degradation profile. In addition, bioelectronic interfaces offer the possibility of integrating electrical stimulation within NGCs to stimulate (and potentially accelerate) *in situ* nerve regeneration. Through regulating neuronal activity, boosting axonal regeneration, and supporting neuroplasticity, electrical stimulation has been demonstrated to stimulate nerve growth. Electrical signals can promote cell proliferation, release neurotrophic factors, and improve the conduit regenerating axon alignment. This controlled stimulation can also support the formation of synaptic connections and improve functional recovery in nerve tissues. Bioelectronic interfaces could be incorporated into NGCs using biocompatible conductive materials, such as poly(3,4-ethylenedioxythiophene) polystyrene sulfonate or graphene, enabling precise control over the electrical signals applied. Therefore, the integration of these technologies promotes the development of highly customizable, multi-functional NGCs that not only provide structural support but also actively participate in the nerve regeneration process.

To further enhance the therapeutic potential of NGCs, developing strategic and personalized conduit selection approaches is essential. Specifically, the structural design of the conduit should be tailored to the length of the nerve defect and the stage of injury. For short-gap defects at an early stage, simple hollow conduits may provide sufficient basic support. In contrast, for long-gap and severe late-stage injuries characterized by scar deposition and reduced SC activity, conduits with stronger guidance capacity and regenerative support, such as multi-channel, porous, or micropatterned designs, are preferred. Moreover, conduit structure should not be considered in isolation, but rather optimized in coordination with material properties, including biocompatibility, mechanical strength, degradation kinetics, and responsiveness to biological signals. Particularly in the context of increasingly functionalized conduit materials, the influence of structural morphology on stem cell differentiation, drug release kinetics, and microenvironment modulation should be given full attention. In terms of fabrication, 3D bioprinting has gradually replaced traditional methods as the mainstream technology for NGC preparation, owing to its capacity for high-resolution and precise reproduction of complex architectures and patient-specific customization. Its advantages are further amplified by the integration of growth factors and bioactive carriers into the printing materials. By leveraging the synergy between structural-material design and the advanced capabilities of 3D bioprinting, next-generation NGCs with multi-functional, intelligent, and personalized features can be developed. Such conduits are not merely passive structural supports, but active participants in modulating the regenerative microenvironment—bridging the gap between structural reconstruction and functional recovery, and moving the field of NTE closer to clinical translation.

In summary, significant progress has been made in the fabrication of NGCs in recent years. As the field of material science and engineering technology continues to innovate, the research and development of NGCs will undoubtedly experience substantial growth. This growth will result in an increasing number of NGCs transitioning from animal experimentation to clinical translation. We believe that by combining the right design, fabrication, materials, and biological factors, the development of NGCs that can effectively repair peripheral nerve deficits is possible within the next decade.

Acknowledgments

The authors would like to gratefully acknowledge De Nayer Stichting for supporting the Advanced Manufacturing Lab.

Funding

The research is funded by the Research Foundation Flanders (FWO) for the doctoral fellowship (1S47325N) granted to Yuexi Zhuang.

Conflict of interest

Eleonora Ferraris is an Editorial Board Member of this journal, but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author contributions

Conceptualization: Yuexi Zhuang
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Writing–original draft: Yuexi Zhuang
Writing–review & editing: Miriam Seiti, Eleonora Ferraris

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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