Review Article

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Material advancement in tissue-engineered nerve conduit

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Abstract: Peripheral nerve injuries resulting from various traumatic events can cause mobility problems and sensory impairment, jeopardizing patients' life quality and bringing serious economic burdens. Due to the shortcomings of autologous nerve grafts, such as limited tissue sources, unmatched size, and loss of innervation at the donor site, tissue-engineered nerve grafts using both natural and synthetic materials have been employed in the treatment of peripheral nerve defect and to promote nerve regeneration. Apart from traditional advantages such as good biocompatibility and controllable degradation, the development of fabrication technology and the advancement in material science have endowed tissue-engineered nerve conduits with upgraded properties such as biomimetic surface topography, extracellular matrix components, neurotrophic factors, and cell seeding, or a conduit with micropores on the surface for substance exchange and/or with fillers inside for microenvironment simulation. This article reviews recent progress in the biomaterials employed in fabricating tissue-engineered nerve conduits, *in vitro* characterization, and their applications in nerve repair in animal studies as well as in clinical trials.

Keywords: tissue-engineered nerve conduit, conduit materials advancement, peripheral nerve regeneration

1 Introduction

Peripheral nerve injury is common in traffic accidents, industrial accidents, and penetrating trauma, which can cause mobility problems, sensory impairment, and even paralysis and seriously affect patients' life quality. Short defect of peripheral nerves is capable of self-regeneration by simple surgical suturing, whereas for a defect distance longer than 3 or 4 times of the diameter, other assistant treatments are essential [1–3].

As is shown in Figure 1, the basic functional unit of the peripheral nerve is the nerve bundle. The endoneurium consists of the endoneurial sheath, basal lamina, and collagen fibers. Single axons enwrapped by endoneurium are grouped into different bundles, surrounded by perineurium [4]. After severe injuries, disruption and necrosis of synapses and axons (Wallerian degeneration) may occur and gradually step into the stage of recovery [5]. Schwann cells are critical for nerve regeneration since they are able to transdifferentiate into repairing phenotype and modulate immune cells such as macrophages to facilitate nerve regeneration. They are also responsible for maintaining the structure of Büngner bands and basal lamina tubes [6,7]. Besides, certain growth factors and their receptors, such as the glial cell line-derived neurotrophic factor family, are also of great importance, and their expression could be upregulated temporarily to provide a favorable microenvironment [8,9].

For minor injuries, peripheral nerve tissue could be sutured directly or connected by fibrin sealant. As the first commercially available biological adhesive product launched in the 1970s, fibrin sealant has been widely used for the seamless repairment of peripheral nerves [10]. The satisfactory therapeutic effect of fibrin sealant

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has been reported in a 70-year-old patient with a 3 cm obturator nerve defect [11]. Studies showed no significant difference between surgical suturing and fibrin sealant in terms of total regenerated axons, fiber orientation, and the recovery of nerve conduction velocity. Apart from the recovery of anatomic integrity, fibrin sealant could also alleviate inflammation and fibrosis, reducing the risk of neuroma and scarring [12]. Moreover, as a user-friendly and time-saving device, fibrin sealant is highly suitable for emergency treatment or inexperienced operators. However, fibrin sealant cannot directly replace the nerve structure; thus, it does not apply to nerve injuries with a long defect, and there remain the risks of infection, necrosis, and seroma [13,14].

As for the lesions that are longer than 3 cm or four times the diameter (critical defect), end to end neurorrhaphy is of no help for the elongation rate and regeneration of nerve fibers [15], and nerve graft transplantation will be essential in order to avoid excessive tension [16]. The traditional gold-standard treatment for the critical defect in peripheral nerves is autologous nerve graft. Allogenic nerve graft is also commonly used in clinical practice. Both methods are disadvantaged in limited tissue sources, unmatched tissue size and structure, loss of innervation at the donor site, and immune rejection towards allogenic grafts [17]. As the most effective treatment, autogenous nerve tissues can provide supportive structure, guidance cues, neurotrophic factors, and Schwann cells without the risk of immune rejection, but complications such as secondary surgery and donor site dysfunction are of significant concern [18,19].

Decellularized allogenic nerve transplantation is an alternative method in which live Schwann cells are removed and only internal structure and crucial extracellular matrix components are maintained. With the maintenance of original structure, decellularized nerve grafts can perfectly guide the oriented growth of regenerating axons [20]. Soon after the implantation of short cell-free nerve allograft, axons, Schwann cells, and inflammatory cells from the host could migrate and gradually reconnect the distal nerve stump, realizing nerve repair and regeneration. Nevertheless, the regeneration potential of long decellularized nerve grafts is impaired because of the lack of Schwann cells [21]. The limited tissue source of human nerves is also a disadvantage. Thus, great efforts have been made to develop tissue-engineered nerve conduits. As the microenvironment factors could significantly modulate the growth of axons inside a nerve conduit, significant progress has been made in exploring new materials, modification of conduit structure and components, as well as nerve repair in animal models and human clinical trials. This article provides a comprehensive review of material advancement in tissue-engineered nerve conduit.

2 Materials for peripheral nerve regeneration

Materials with various properties in favor of nerve regeneration have been developed in the past decades, such as suitable mechanical strength, excellent biocompatibility, modulated degradation profile, and the capacity to encapsulate cells, growth factors, and bioactive proteins. Biomaterials for peripheral nerve repair can be generally divided into natural materials and synthetic materials in terms of their origins. Natural materials are represented by human cadaver, or animal-derived nerve grafts (autologous, allogenic, or xenogeneic nerve grafts), nonneural tissues (blood vessels, tendons, muscles, and outer membrane sheaths) [22–27], and biological macromolecules and their derivatives. However, immune rejection against allogenic and xenogeneic grafts is a major problem that restricts their application [28]. Biological macromolecules used as natural materials are primarily the extracellular matrix components with guaranteed biocompatibility [29]. Somehow, synthetic materials are more frequently used in *in vivo* studies due to their excellent mechanical properties and modulated degradability. In line with the original structure of nerve bundles, the tubular conduit has been considered the best physical format because it can minimize surgical trauma and store up neurotrophic factors released from the posttraumatic neural stem to promote nerve regeneration. Different manufacturing processes, including low-temperature deposition, electrospinning, leaching, 3D printing, lyophilization, and casting, have been employed to fabricate nerve conduit according to respective properties of different materials [30–37].

3 Natural materials

3.1 Silk fibroin (SF)

Natural silk is one of the natural macromolecular proteins, consisting of sericin and two silk fibroin monofilament fibers wrapped in the inner layer. Silk fibroin is a natural polymer composed of 20 amino acids, among

which glycine, alanine, and serine are the main components [38]. As a major component of natural silk fiber, silk fibroin has been widely used in regenerative medicine in different forms for its self-assembly characteristics [39–43]. Silk fibroin has expanded the surface to promote cell adhesion, proliferation, migration, and nutrient diffusion [44,45]. Silk fibroin could improve the process of angiogenesis inside a conduit due to the stimulation of fibroblast proliferation and production of vascular endothelial growth factor, which was proven to benefit nerve regeneration [46]. As early as in 2006, Gu's group demonstrated that silk fibroin was biocompatible with nerve cells and nerve tissues such as Schwann cells and hippocampal neurons [47,48]. They assembled a novel conduit with silk fibrin wall and oriented silk fibroin fibers inside to bridge over a sciatic nerve defect of 10 mm *in vivo*. The diameter and thickness of silk fibroin conduits were customizable with a stainless-steel casting mold fixed on the mold bottom. Furthermore, the lumen of the conduit was filled with 20 longitudinal-aligned silk fibroin fibers. As the silk fibroin material was covered with interconnected pores, this eggshell-like structure exhibited good mechanical stability and permeability. At six months post-operation, the silk fibroin conduit was replaced by nerve-like tissues without inflammatory reaction. And locomotor functional recovery of injured limbs was comparable to that of autologous nerve transplantation [49]. In addition to the conduit itself, the constructed microenvironment where normal peripheral nerve tissue could grow can promote nerve regeneration [50]. The same team also introduced a nature-mimicked microenvironment for nerve regeneration by coculturing dorsal root ganglia and Schwann cells *in vitro* and then seeded the cell mixture into a silk fibroin conduit. When bridging a 10 mm-long sciatic nerve defect in rats, they found that at the early stage (4 weeks), expression of nerve regeneration-related protein (N-cadherin and PMP22) in the silk fibroin-based conduit group was higher than that of control groups. At the late stage (12 weeks), the conduit produced a further improved outcome of nerve regeneration and functional recovery (walking track analysis), which was close to that of autologous transplantation [51]. Although native silk possesses good mechanical properties, when degummed, silk fibroin takes on impaired mechanical properties, and thus compromising the optimal properties for nerve conduit when using silk fibroin materials.

3.2 Collagen

As the main component of connective tissue and extracellular matrix, collagens are the most abundant proteins in mammals (30% of total protein mass), consisting of various glycoproteins synthesized by fibroblasts [52]. Moreover, collagen is characterized by a triple helix structure formed by combining three polypeptide chains, where several lysine can be detected for further modification [53]. Thus, collagen is one of the optimal biomaterial types that are widely used in regenerating and reconstructing both soft tissues and hard tissues in various forms [54–57]. In nerves, type IV procollagen produced by Schwann cells is the major structural component of both endoneurium and perineurium. Compared with synthetic polymers, biomaterials made of collagen tend to have better biocompatibility and less immunological rejection.

Keilhoff *et al.* employed collagen type I/III conduit to repair rat sciatic nerve defects with 4 mm length. Seven days after implantation *in vitro*, the scanning electron microscopy showed that Schwann cells maintained their typical shape. Abundant regenerated Schwann cells on the internal surface of collagen conduits and the formation of Büngner band were observed, and collagen conduits could be well-integrated with host tissues and fully revascularized in 5–7 days. During the revascular process, macrophages were also timely recruited to the reparative site to perform their immunological and regenerative roles. There was no resorption of the conduit after three weeks; thus, this conduit can be a candidate for tissue-engineered conduits [58]. The collagen conduits seem to be suitable for different lengths and sites of the various peripheral nerve defects. It was reported that collagen I/III conduits were of equal therapeutic efficiency to the golden standard in repairing 10 mm-long peroneal nerve defect in rat models. At 12 weeks post-operation, the whole conduits were resorbed, and nerve-like tissues could be observed. Compared to the control group (severed and no treatment), there was an increased number of small axons in the collagen conduit group, and the mean axonal loss per rat was significantly lower than that of the control group [59]. Besides, collagen is unstable under an aqueous environment and may become swollen and be dissolved gradually [44]. Thus, a combination of collagen with other materials is likely to improve its performance and expand its application of collagen conduit in nerve regeneration.

3.3 Chitin/chitosan

Chitin is the second most abundant polysaccharide on earth, only after cellulose. It exists widely in crustaceans, insects, or cell walls of fungi [60]. Chitosan is the byproduct

of *N*-deacetylated chitin that can degrade into glucosamine and *N*-acetyl glucosamine in the lysosome [61,62]. As a natural material whose degradation rate can be regulated by deacetylation, chitosan is an ideal candidate for artificial nerve grafts with excellent biocompatibility, permeability, and plasticity [63,64]. Besides, chitosan is characterized by low production cost, easy access, and easy processing. Moreover, the intermediate product – chitooligosaccharides – is also able to promote axon regeneration by inhibiting neuronal apoptosis and facilitating cell adhesion [65,66].

Tissue fibrosis is partially responsible for the failed repair of injured peripheral nerves. One study has demonstrated that chitosan membranes could alleviate this process by reducing the infiltration of fibroblasts [67]. Some researchers began to study the role of chitosan in nerve regeneration using large animal models. Over a follow-up of 6 months, researchers employed chitosan conduit to repair sciatic nerve injuries (3 cm) in dog models. The dogs in the conduit group and autologous nerve graft group could walk and run freely at six months after surgery, and their posture tended to be normal. However, dogs in no treatment group suffered from skin ulcer and muscle atrophy and disability to use the repaired leg. And no rejection was observed in the chitosan group. Slower than normal nerves, the conduction velocity of the chitosan conduit group and the autologous group were comparable. Also, no edema, hematoma, inflammation, or abscess were found in the conduit group. The thickness of regenerated nerve in chitosan conduits was similar to that of the autologous group. In contrast, no regenerative nerve could be observed in the control group, indicating that the chitosan conduit could bridge the gap between the nerve stumps and stimulate nerve regeneration [68].

Some researchers managed to enhance the mechanical property and tensile strength of chitosan conduit by adding chitin powder to the chitosan solution [69].

In vivo implantation showed that the conduits fabricated by modified chitosan remained biocompatible to surrounding host tissues [70]. Chitosan could also be upgraded by combining with other bioactive substances or materials. Zhang *et al.* seeded mesenchymal stem cells (MSC) on erythropoietin (EPO)-loaded chitosan nerve conduit (EPO/Chi) by mixing Matrigel with MSCs, then injected the mixture into the conduits after their implantation. *In vitro* experiments showed improved cellular viability and migration of mesenchymal cells and Schwann cells as well as their neurotrophins secretion. According to silver staining, after 8 weeks, the number of axons in the injured nerve bundles increased significantly in Gel/MSC than in the hollow conduit group. In addition, the level of morphological restoration and regularity of nerve bundles also showed better results than the hollow conduit [71]. Undeniably, chitin and chitosan have been widely applied, but several drawbacks limit their scope of application, such as the risk of allergy to seafood and dependence on the low-pH environment.

4 Synthetic materials

4.1 Silicone

Silicone is characterized by nontoxic and bioinert characters, which does not react with any substances except for strong acid and alkaline and thus is nonabsorbable and non-deformable. Thanks to its nonporous structure, silicone cannot harbor bacteria internally and is easily sterilized [72]. Hollow or nonporous designs are the most primitive of all conduit designs. Francel *et al.* compared the performance of empty silicone conduits with the silicone conduits containing a short-inserted nerve segment, and with nerve autografts in repairing sciatic nerve defects (13–15 mm), the conduction velocity of silicone conduit with nerve segment group was comparable to that of the autologous group and showed signs of nerve regeneration by a large number of myelinated axons. Moreover, walking track analysis with sciatic function index (SFI) value calculation exhibited no significant difference between silicone conduit with nerve segment group and autologous group. On the contrary, in the hollow silicone conduit group, the chamber was blocked by the serum, and no functional recovery could be observed [73]. Thus, silicone conduits are often loaded with various growth factors, cells, and other materials to promote their performance [74–76].

Although readily available, silicone cannot replace tissue transplantation in patients for extended nerve defects as it can block the interaction between cells inside and microenvironmental factors outside. Besides, since silicone is nondegradable *in vivo*, neuroma formation and nerve dysfunction induced by foreign body reaction and compression of neo-tissues may cause local discomfort and lead to secondary operation to remove the implant.

4.2 Polytetrafluoroethylene (PTFE)

As another bioinert synthetic material that has been widely applied in nerve repair, polytetrafluoroethylene

(PTFE) is of high surface tension and viscosity resistance. Because of the chemical inertness, its modification was difficult by surface coating and thus has been a scientific and technological challenge for decades [77]. When PTFE conduits were applied to repair a 10 mm-long nerve defect, Zetti *et al.* introduced a novel PTFE conduit with porous structure with various sizes by extrusion and mechanical stretching. Six months after the surgery, all the rats were reinnervated on the operated side, according to electromyographic studies. The process of axonal regeneration tended to be significantly active because histological tests can observe thinly myelinated fibers and medium-large clusters, thus confirming the feasibility of nerve regeneration with PTFE-based conduits [78]. However, there was no control group in this study, and thus the results were less reliable. Labroo *et al.* also reported a GDNF-embedded PTFE conduit for nerve repair using a GDNF reservoir between two PTFE conduits. They found that the drug release rate could be adjusted by the size and quantity of the pores. Afterward, this GDNF-embedded nerve conduit was used to reconnect sciatic nerve defect of 10 mm length in BL6 YFP mouse models. Compared with using PTFE conduits alone, at 10 weeks after surgery, the distal nerve in the GDNF conduit group revealed a significantly higher number of myelinated axons. Additionally, at 18 weeks postimplantation, the GDNF conduit groups had significant myelination with healthier-looking nerve surrounded by multiple fibrous layers [79]. Same as silicone conduit, PTFE conduits can hardly break down *in vivo*, which may trigger persistent rejection reaction and hinder the general repair process. Generally speaking, the development of nondegradable guide conduits has not been actively pursued due to the limitations of permanent material [80].

4.3 Polyglycolic acid (PGA)

Polyglycolic acid (PGA) is a rigid thermoplastic polymer with features of high crystallinity, high tensile modulus, and low solubility in organic solvents. PGA retains both hydrophilic and lipophilic within their repetitive units, allowing processing and modification into different forms without additives [81]. Because of the nonspecific fracture of the ester chain, PGA materials could lose their strength (1–2 months) and total mass (6–12 months) fast *in vivo* [82,83].

The sciatic nerve has been extensively studied because of easy access and evaluation, whereas some of the nerves are more difficult to reach functional regeneration after injury, such as the recurrent laryngeal nerve (RLN), and thus are rarely reported. Sentürk *et al.* used a PGA conduit to repair damaged RLN, and they found that the vocal cord mobility was proportionally higher in the PGA conduit group than in other groups (only nerve cuts and primary repair group). In fact, there was a positive relationship between axon number and vocal cord mobility. And the mean number of axons in the PGA conduit group was the highest among the three groups. Histological evaluation outcomes indicated that this conduit has the potential to repair peripheral nerve damage by creating an isolated environment, then provide a more accurate orientation of nerve fibers [84]. Nevertheless, it was observed that PGA conduit might cause significant foreign body reaction and inflammation likely caused by rapidly degraded products [84].

4.4 Polyurethane (PU)

As one of the most versatile polymers, polyurethane (PU) accounts for 6% of the world's plastic use [85]. PU is produced by the polyaddition reaction of long-chain oligomer diol with diisocyanate. The typical microphase-separated structure of the hard segment (diisocyanate and chain extender) and soft segment (long-chain diol) or oligodiols endows perfect mechanical properties such as wear resistance, elasticity, and high tensile strength. Moreover, PU could be fabricated into various hardness and different forms by modulating its cross-link density and molecular structure, resulting in regulated mechanical properties and permeability [86]. *In vivo* studies also found that PU could promote human umbilical vein endothelial cells' adhesion and resist hemolysis and clotting [87], making it a promising material for regenerative medicine. In 2015, Wu *et al.* reported that PU could significantly improve Schwann cells' myelin-related gene expression and neurotrophin secretion, which suggested that PU could serve as a promising candidate for peripheral nerve regeneration [88]. Meanwhile, a single PU nerve conduit with a pore size of 1–5 mm and a porosity of 88% was investigated to repair a 10 mm nerve defect. When cultured *in vitro*, the glial cell could well grow on PU conduits, indicating good cytocompatibility of PU conduits. After *in vivo* implantation, no inflammatory or adverse tissue reaction was observed. Furthermore, the regenerated neurofilaments were found distributed regularly inside the PU conduit, similar to the pattern of the autologous group. At different time points postsurgery (2, 4, 8, 10, 14, and 20 weeks), functional nerve recovery was observed by walking track analysis, which showed that

the PU conduit group exhibited a higher SFI value than that of PCL and silicone groups, revealing a better reparative capability [89].

4.5 Poly(L-lactide-*co*-caprolactone) (PLCL)

PLCL is an aliphatic polyester copolymer prepared by ring-open polymerization of poly(L-lactic acid) (PLLA) and poly(e-caprolactone) (PCL). The strength and degradation rate of PLCL can also be modified by changing the proportion of PLLA and PCL. The copolymer PLCL made up for the brittle behavior of PLLA and the low stiffness of PCL and improved the local acidic environment caused by PLLA degradation [90]. PLCL can be customized for desired degradation rate by changing the ratio of PCL/PLLA, and the more PLLA, the higher the degradation rate [91]. Moreover, when the content of PCL is increased, the shape memory effect of PLCL will also be improved [90].

As early as 2009, electrospun nanofibrous substrates of PLCL have been reported to promote mesenchymal stem cell differentiation into neuronal cells for nerve tissue engineering [92]. It was recently demonstrated that modified PLCL conduits could significantly improve SC migration, proliferation, and myelination, promote the differentiation of PC12 cells (exhibiting many neuronal properties), and upregulate the expression of neurite growth-related proteins *in vitro* [93]. *In vivo* studies further proved that the PLCL conduit could fully restore motor function (evaluated by triceps surae muscles weight ratio) and nerve conduction function (electrophysiological analysis) in 12 weeks, and the therapeutic effect was equal to that of the gold-standard treatment (autologous transplantation) [93]. Moreover, compared with the electrospun caprolactone group, PLCL conduit had a lower risk of neuroma formation or infection [94]. However, PLCL is highly hydrophobic and lacks cell recognition sites, and thus causes low biocompatibility [95]. Enhancement in the topological modification of PLCL to increase the cytocompatibility and cell adhesion remains a challenge for researchers in the future.

4.6 PLLA

Polymers of lactic acid (PLA) can be decomposed into glycolate and pyruvate and hydrolyzed into H_2O and CO_2 eventually *in vivo*. Owing to the asymmetric carbon atom in the lactic acid molecule, PLA is featured with optical activity and composed of D-type (PDLA, right-handed rotation) and L-type (PLLA, left-handed rotation) [96]. Both PLA and PLLA are widely applied in the field of tissue-engineered repair [97]. The amorphous areas of PLLA usually degrade faster, leading to the increase of crystal concentration of the rest materials that become resistant to degradation. Therefore, they may remain in the body for a long time accompanied by inflammatory cells [98].

As a plastic material that is easy to obtain and process [99], a single PLLA conduit was implanted in Sprague Dawley rats to repair a defect of 12 mm in the right sciatic nerve. Evaluation of long-term (8 months) recovery showed that the PLLA conduit could maintain the structural integrity and induce vascularization. The SFI value demonstrated no difference between the autologous graft group and the PLLA conduit group. Besides, the number of axons per unit area in the PLLA conduit was comparable to that in isograft controls, suggesting that the PLLA conduit might be used as a suitable conduit for nerve regeneration [100]. The same team reported similar treatment outcomes in another study where allogeneic Schwann cells were seeded onto the PLLA conduit to facilitate nerve repairing [101]. It could cause some problems such as incomplete degradation and decreasing pH values as a relatively slow-degradation material. Thus, PLLA implant may incur late-phase inflammation, cell necrosis, and delayed axon recovery *in vivo* [98].

4.7 Poly(lactic-*co*-glycolic acid) (PLGA)

PLGA is one of the most popular biodegradable copolymers, consisting of two different monomers – polylactic acid and polyglycolic acid and it can be synthesized by ring-opening copolymerization in the presence of catalysts [102]. Several factors can affect the degradation of PLGA copolymers, such as molecular weight, crystallinity, and the ratio of lactic acid to glycolic acid. Generally, PLGA degrades faster when the glycolic acid part increases (<50%) in the main chain [103,104]. As a biosafe material that can promote the formation of capsules and film, PLGA has been widely used in the pharmaceutical and tissue engineering industry [105]. Researchers found that PLGA membrane pre-coated with Nectin-like molecule 1 (NECL1) could mimic natural axons to improve the adhesion of Schwann cells. *In vitro*, they compared the single PLGA conduit with the PLGA conduit coated with NECL1 of different concentrations (PLGA/NECL1)

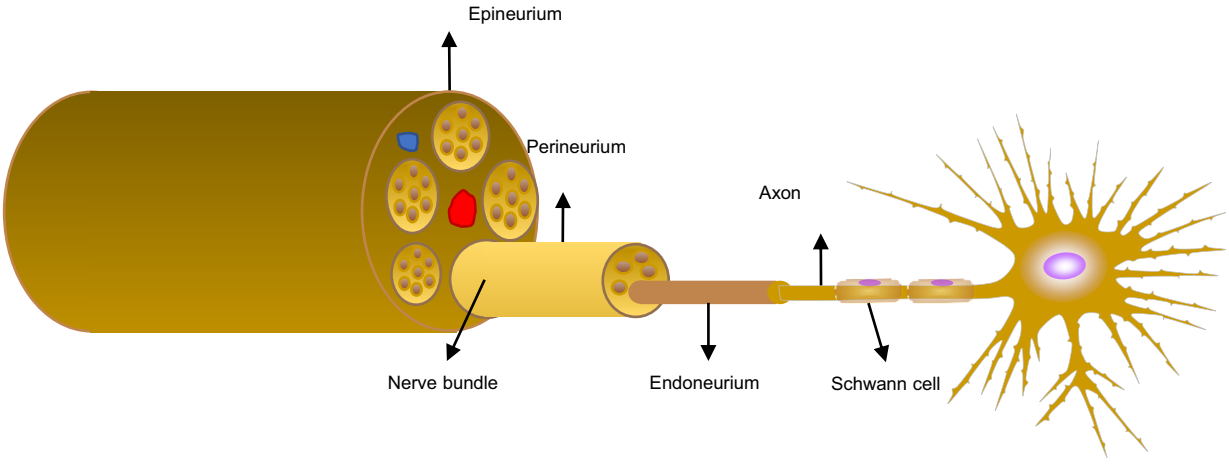

Figure 1: The anatomy of peripheral nerves.

and observed a significant increase in the gene expression of neurotrophic factors related to nerve regeneration in the PLGA/NECL1 group (the concentration of 50 ng/mL had the best performance). Moreover, MTT assay showed that the PLGA/NECL1 conduit could also better promote cell viability and proliferation. *In vivo*, they evaluated nerve conduction velocity and muscle weight ratio among single PLGA/NECL1 group, PLGA conduit group, and transected group, and they found that the PLGA/NECL1 group was better-performed over the rest two control groups (single PLGA and transected group). SFI results also indicated that the PLGA/NECL1 group had a faster recovery by 12 weeks. Thus, they concluded that PLGA conduit pre-coated with NECL1 could facilitate the regeneration of peripheral nerves [106].

However, when applied *in vivo*, the relatively low mechanical properties and non-bioactive nature are the apparent drawbacks of pure PLGA conduit [107]. Different modifications have been incorporated into PLGA to create polymer composites with desirable properties to overcome these disadvantages. Hou *et al.* tried to promote the repairing effect of PLGA conduit by combining the electrospinning PLGA membrane and freeze-dried oxidized antibacterial cellulose and collagen sponge. The ECM-like sponge was expected to facilitate tissue regeneration, while the external PLGA conduit could bridge the defect and provide mechanical support. The conduits were implanted in rats with a 10 mm defect in the sciatic nerve. Staining results demonstrated that the number of nerve fibers and blood cells/vessels in modified conduit groups was significantly higher than that of hollow conduit groups and exhibited uniform and orderly distribution. By 8 weeks postoperation, the positive expressions of proteins related to nerve regeneration (S-100 and NF-200) in modified conduit groups were close to those of autologous groups and much higher than those of hollow conduit groups [108].

4.8 Composite materials

As described, natural substances are good in biocompatibility but relatively weak in mechanical properties, whereas synthetic materials are the opposite. Composite materials have been developed and evolved to make the best use of their prominent features. It was believed that composite materials are a promising alternative to traditional nerve conduits in the future.

Composite materials were fabricated initially by simple mixture of various components. Nieto-Suarez *et al.* observed that the porous chitosan/gelatin conduit prepared through ice segregation-induced self-assembly (ISISA) was stable and highly swollen, suitable for fabricating tissue-engineered nerve conduits [109]. Some researchers used one material as the major and modified it with other materials. Ouyang *et al.* invented a seamless axially aligned nerve graft composed of collagen (25%) and PLGA nanofibers by a one-step modified electrospinning technique. With the application of insulating mandrel of different sizes, the fibers on both inner and outer walls were aligned along the longitudinal axis, and the inner diameter of the scaffold could be adjusted from 1 to 10 mm. After being seeded into the conduits *in vitro*, Schwann cells in conduits resembled a morphology similar to that of normal nerves, indicating good biocompatibility of the conduit. The gastrocnemius muscle cross-sectional and SFI values in both autograft and the uniaxially aligned seamless collagen/PLGA conduit groups were larger and higher than those of randomly oriented groups. HE staining analysis also demonstrated well-organized structures of

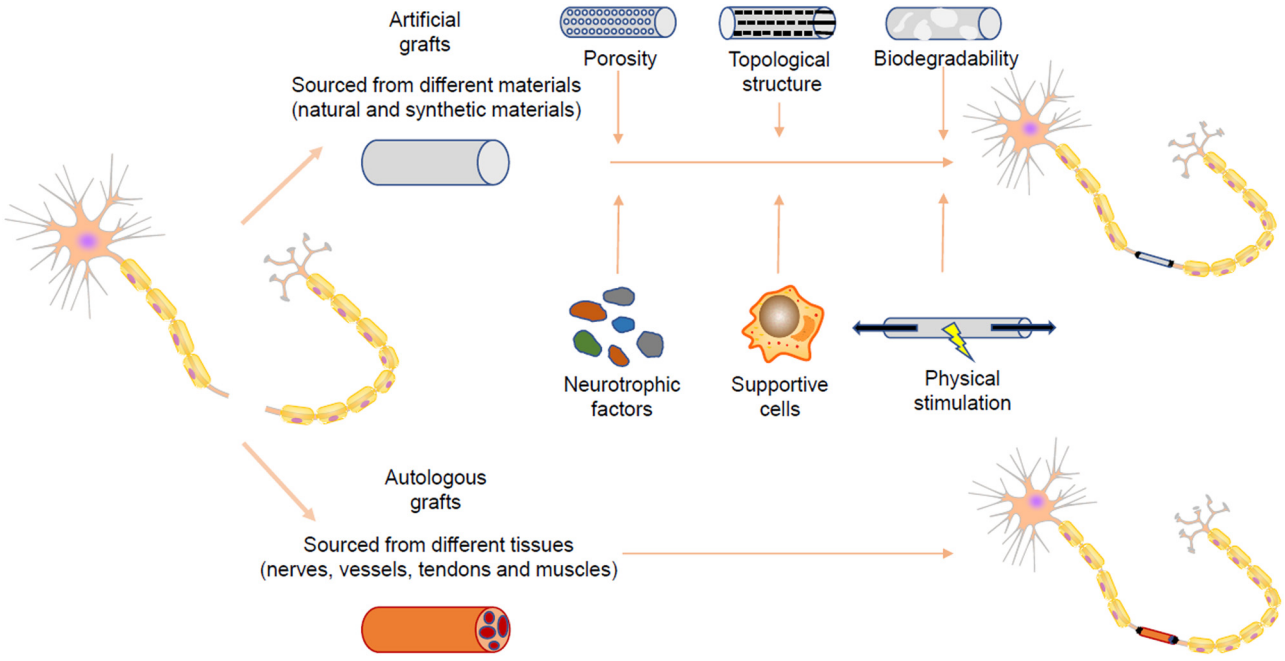

Figure 2: Schematic illustration of peripheral nerve repair strategies *via* tissue-engineered conduit materials.

regenerated axons, indicating a good reparative effect of uniaxially aligned seamless collagen/PLGA nanofibers [110].

Researchers started making new tissue-engineered nerve conduits with three or more bioactive materials combined using advanced fabrication technology based on a better understanding of nerve regeneration mechanisms. Wang *et al.* developed a novel PLGA-silk fibroin-collagen (PLGA-SF-COL) conduit with improved hydrophilicity and mechanical property. When cultured for 3 days on the conduits, Schwann cells revealed good cytocompatibility to the conduit as they attached well and exhibited the normal phenotype (bipolar or tripolar morphology). MTT assay also showed higher value in PLGA-SF-COL conduit than that in the pure PLGA conduit group. They concluded that PLGA-SF-COL conduits composed of 30% PLGA, 35% silk fibroin, and 35% collagen had the potential to facilitate nerve regeneration. However, they only conducted cytology experiments *in vitro*; studies *in vivo* need to be further validated [111] (Figure 2).

4.9 Clinical trials

As research continues, several conduits have been applied in clinical trials and exhibited promising outcomes (Table 1 and Figure 3). When the nerve defect was minor, the microsurgical suture is the first choice in clinical treatment. As early as in 1982, Lundborg *et al.* reported a clinical randomized prospective study of repairing median and ulnar nerve defect with 3–5 mm length. The study followed 28 patients for up to five years to compare the repair outcome of tubular treatment (silicone conduits) with traditional microsurgical repair. After 5 years, they found no statistical differences in clinical or neurophysiological outcomes of tubular repair and the traditional microsurgical treatment. Moreover, cold intolerance was much better relieved in the tube group than in the microsurgical suturing group. Therefore, they concluded that tubular treatment could be an alternative for traditional microsurgical repair [112].

The collagen-based conduit was first applied for the clinical repair of brachial plexus nerve injuries in 2006. They defined the motor scale composite (MSC) as a numeric measure for representing the brachial plexus palsy motor outcome. And MSC value ≥0.6 represents good functional recovery and ≥0.75 represents an excellent functional recovery. Finally, three patients (total of 5) exhibited an excellent recovery (MSC ≥0.75), and one reached a good recovery (MSC ≥0.6) at 2 years postoperation. In all five patients, applying collagen-based conduits alone could improve the MSC value by 69 and 78% at 1 and 2 years, respectively. Moreover, joint movement scores of four patients also improved to a range of motion of more than 56%. Overall, most patients achieved a good functional recovery with improved functions of feeding and dressing by themselves [113].

Table 1: Clinical studies of materials employed in periphery nerve repairs

Materials	Nerves	Length (mm)	Outcomes	Reference
Silicone	Median and ulnar nerve	3–5	Comparable therapeutic effects (except for cold intolerance) to that of the traditional microsurgical treatment (direct suture)	[112]
Collagen	Brachial plexus nerve	≤20	MSC ≥ 0.6 in 4/5 cases	[113]
PTFE	Median and ulnar nerve	15–60	15–40 mm: functional recovery of both motor and sensory nerves in 78.6% cases (15 of 17 median nerves and 7 of 11 ulnar nerves); 41–60 mm: functional recovery in 13.3% cases (1 of 4 median nerves and 1 of 11 ulnar nerves)	[114]
	Inferior alveolar and lingual nerve	2–15	Functional recovery in 2/7 cases (2 inferior alveolar nerves <3 mm)	[115]
Polyglycolic acid (PGA)	Digital nerve	5–30	Excellent functional sensation recovery in 33% cases and good in 53% cases; pain sense recovery in over 77% cases	[116]
	Inferior alveolar and lingual nerve	/	Allodynia or dysesthesia resolved in 6/10 cases (3 inferior alveolar nerves and 3 lingual nerves); sensory functional recovery in all cases; reconnection with normal nerve-like structure in 9/10 cases	[117]
PGA and collagen	Frontalis branch of facial nerve	11 mm and 28 mm	Partial muscle contraction and recovered distal latency in 1/2 cases (by 2 months); symmetrical eyebrow-lifting in 2/2 cases (by 5 months)	[118]

"/": The length was not mentioned in the original articles.

Stanec *et al.* describe the effectiveness of expanded-polytetrafluoroethylene (e-PTFE) conduits in the clinical repair of nerves in the upper limbs. In the study, all the patients were evaluated and classified into 2 groups (minor defect and major defect). In the minor group (1.5–4 cm defect), most (78.6%) of the patients showed functional recovery of both motor and sensory nerves (15 of 17 reconstructed median nerves and 7 of 11 reconstructed ulnar nerves), whereas in the major group (4.1–6 cm defect), only a minority (13.3%) of the patients achieved functional recovery (1 of 4 reconstructed median nerves and 1 of 11 reconstructed ulnar nerves). These results concluded that long defect nerve injury remains a challenge to PTFE conduit, although it could be a measure to repair median or ulnar nerve defects shorter than 4 cm [114].

However, others reported that successful repair (sensation restoration) of inferior alveolar nerve and lingual nerve *via* Gore-Tex tubing (e-PTFE) was only observed in two of seven patients whose nerve injuries were shorter than 3 mm. One patient was fully recovered, and the other was close to expected recovery; the rest cases showed compromised sensational restoration after 3 years. It is known that inferior alveolar nerves tend to have a higher spontaneous recovery rate than lingual nerves. In this study, the only two cases succeeded in repairing are both inferior alveolar nerves shorter than 3 mm and thus the results could hardly indicate that Gore-Tex tubing was capable of repairing nerve defect [115].

As early as in 1985, researchers applied PGA conduits to reconstruct nerve defects with an averaged defect of 1.7 cm (from 0.5 to 3.0 cm) in 15 patients with a follow-up evaluation of sensibility for an average of 22.4 months. Consequently, 33% achieved excellent functional sensation and 53% achieved good functional sensation in their evaluation according to sensory relearning exercise. Also, over 77% of patients who lost their pain feeling before the surgery could achieve good repair, which revealed a satisfactory repair outcome [116].

Several composite materials have already been applied in clinical trials too. Seo *et al.* reported that PGA conduits coated with collagen (PGA-c) were applied to bridge lingual nerve or inferior alveolar nerve in a university hospital, and the outcomes were evaluated at 2 months to 8 years postsurgery. Allodynia or dysesthesia was resolved in 60% (6/10) and greatly reduced in 40% (4/10) of the patients. In almost all patients, functional sensory recovery can be observed. Thus, the results demonstrated that PGA-c conduits could improve the patients' condition according to quantitative sensory

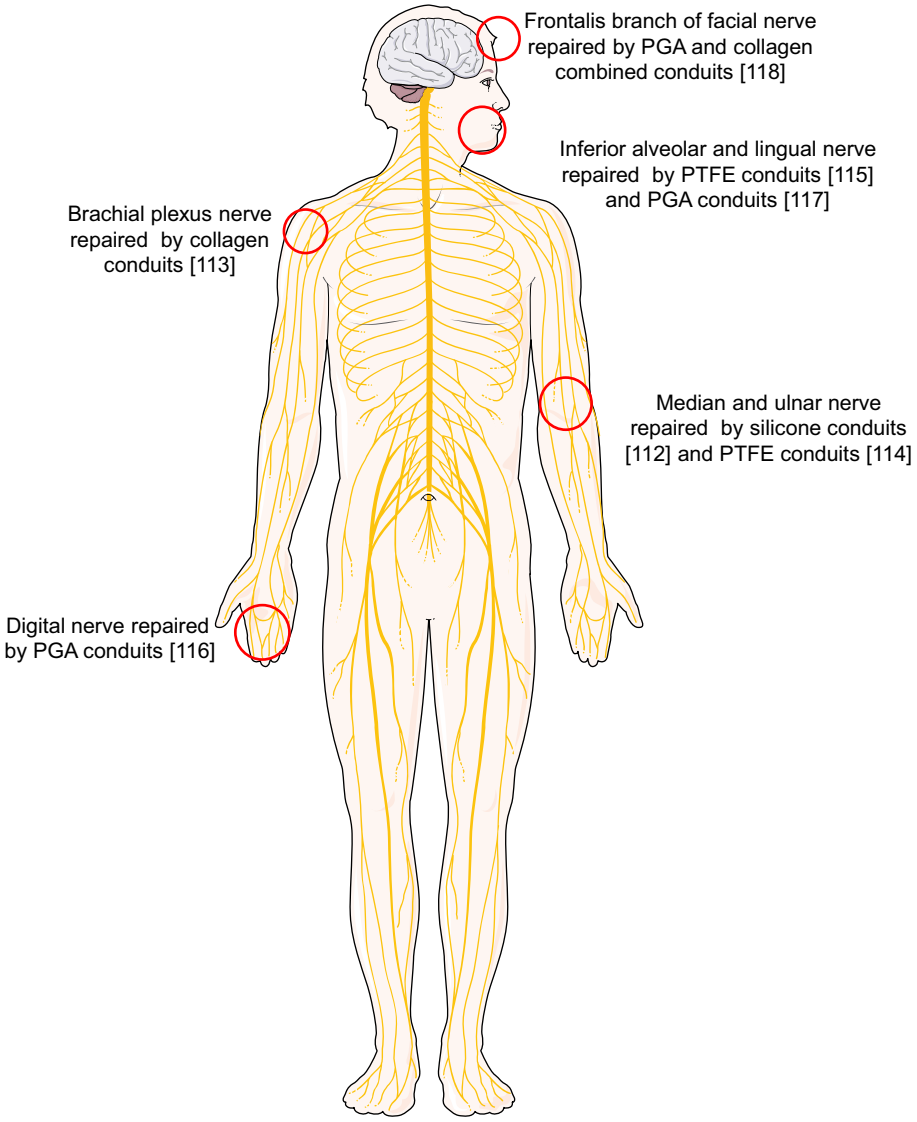

Figure 3: Schematic illustration of clinical trials of peripheral nerve repair with different types of nerve conduits at different anatomic regions.

testing results (brush stroke perception, mechanical touch threshold, sensitivity to cold/hot stimuli). And normal nerve-like structure could be observed in 9/10 cases, which indicated PGA-c conduits as a promising way for nerve regeneration [117]. Inada *et al.* reported that cylindrically woven PGA conduit filled with collagen was applied to repair peripheral motor nerve defects in two patients. Two months after the operation, partial contraction of the left frontalis muscle could be observed in one patient. Electrophysiological testing showed that the distal latency of both patients recovered and became almost symmetrical with the normal side. Five months later, both patients could manage to lift their eyebrows symmetrically with full recovery [118].

5 Conclusion

The recovery of injured periphery nerve highly relies on the structural integrity and functional restoration of nerve conduit. As is shown in Tables 1 and 2, advances in biomaterials have greatly contributed to the development of tissue-engineered nerve conduit and periphery nerve regeneration in the past decades. With upgraded biosafety and mechanical features, tissue-engineered nerve conduit has been evolved from a single hollow tube that merely connects both ends to the composite structure with multiple layers and a delicate topological surface for exchanging substances and regulating cell behaviors. Accumulated studies *in vitro* and *in vivo* have also

Table 2: *In vivo* studies of materials employed in periphery nerve repairs

Materials	Objects	Nerves	Length (mm)	Outcomes	Reference
Silk fibroin	SD rats	Sciatic nerve	10	Replaced by nerve-like tissues in 6 months	[49]
	SD rats	Sciatic nerve	10	By 4 weeks (early stage): increased expression of nerve regeneration-related proteins By 12 weeks (late stage): densely distributed myelinated nerve fibers and muscles with large cross-sectional area	[51]
Collagen	SD rats	Sciatic nerve	4	Schwann cells regeneration, Bü-ngner band formation, and hematogenous macrophages recruitment in 5–7 days	[58]
	SD rats	Peroneal nerve	30–40	Increased number of small axons and nerve-like tissues by 12 weeks	[59]
Chitosan	Dog	Sciatic nerve	30	Comparable motor recovery (walk, run, posture), conduction velocity, and thickness of regenerated nerves to that of the autograft group	[68]
	SD rats	Sciatic nerve	5	Significantly improved axon density, morphological restoration, and nerve bundle distribution in both the conduit + Gel/MSC group and the conduit group by 8 weeks	[71]
Silicone	Lewis rats	Sciatic nerve	13–15	Increased number of myelinated axons; comparable conduction velocity and SFI value to that of the autograft group	[73]
Polytetrafluoroethylene (PTFE)	Wistar rats	Sciatic nerve	10	The operated side was reinnervated in all cases by 6 months	[78]
	BL6 YFP mouse	Sciatic nerve	10	Increased number of myelinated axons in the GDNF conduit group by 10 weeks; healthier-looking nerves and significant myelination in the FK506 group and the GDNF conduit group by 18 weeks	[79]
Polyglycolic acid (PGA)	Wistar rats	Recurrent laryngeal nerve	/	Accurate orientation of nerve fibers, enhanced vocal cord mobility, and increased number of axons in the PGA conduit group	[84]
Polyurethane (PU)	SD rats	Sciatic nerve	10	End-end connection of regenerated nerves, regularly distributed neurofilaments, and an obviously higher SFI value in the PU conduit group	[89]
Poly(L-lactic-*co*-ε-caprolactone)	SD rats	Sciatic nerve	12	Improved SC migration, proliferation, myelination, and upregulated expression of neurite growth-related proteins; nerve conduction and motor function were restored in all cases by 12 weeks; comparable therapeutic effects to that of the autograft transplantation	[93]
Poly(L-lactic acid) (PLLA)	SD rats	Sciatic nerve	10	Promoted vascularization and axon density	[100]
Poly(lactic-*co*-glycolic acid) (PLGA)	SD rats	Tibio-peroneal bifurcation	12	Promoted cell proliferation and neurotrophic factor levels in the PLGA/NECL1 group; sciatic nerve stumps were reconnected in the PLGA/NECL1 groups by 12 weeks	[106]
PLGA, oxidized bacterial cellulose and collagen	SD rats	Sciatic nerve	10	Increased number of myelinated nerve fibers and blood cells/vessels; comparable SFI value to that of the autograft group	[108]
PLGA and collagen	SD rats	Sciatic nerve	15	Better SFI recovery and larger gastrocnemius muscle cross-sectional area in the uniaxially aligned collagen/PLGA conduit group than in the randomly oriented collagen/PLGA conduit group	[110]

"/": The length was not mentioned in the original articles.

confirmed the facilitating effects of newly developed materials in nerve regeneration, some of which have even been testified in clinical trials with satisfactory outcomes. In the future, tissue-engineered nerve conduits are expected to self-adjust to their *in vivo* complex microenvironments where the conduits are implanted [119]. Moreover, upgraded materials should actively participate in the repairing process by modulating different cells (Schwann cells, stem cells, and immune cells) and neurotrophic factors [120–122] and responding to physical stimulation as well as electrophysiological signals to accelerate nerve restoration from all aspects. With the further research of biomaterial science by combining fabrication and manufacturing technologies with chemical components and biological mechanisms, clinical application of tissue-engineered conduit will be a promising way of peripheral nerve regeneration.

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