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Biomimetic neural scaffolds: a crucial step towards optimal peripheral nerve regeneration

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Abstract

Peripheral nerve injury is a common disease that affects more than 20 million people in the United States alone and remains a major burden to society. The current gold standard treatment for critical-sized nerve defects is autologous nerve graft transplantation; however, this method is limited in many ways and does not always lead to satisfactory outcomes. The limitations of autografts have prompted investigations into artificial neural scaffolds as replacements, and some neural scaffold devices have progressed to widespread clinical use; scaffold technology overall has yet to be shown to be consistently on a par with or superior to autografts. Recent advances in biomimetic scaffold technologies have opened up many new and exciting opportunities, and novel improvements in material, fabrication technique, scaffold architecture, and lumen surface modifications that better reflect biological anatomy and physiology have independently been shown to benefit overall nerve regeneration. Furthermore, biomimetic features of neural scaffolds have also been shown to work synergistically with other nerve regeneration therapy strategies such as growth factor supplementation, stem cell transplantation, and cell surface glycoengineering. This review summarizes the current state of neural scaffolds, highlights major advances in biomimetic technologies, and discusses future opportunities in the field of peripheral nerve regeneration.

Graphical abstract

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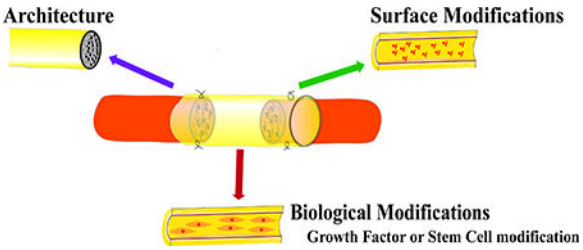
Conflicts of interest

There are no conflicts to declare.



1. Introduction

Peripheral nerve injury (PNI) is a common disease due most frequently to trauma and can also be caused by congenital defects or surgical procedures. The National Institute of Neurological Disorders and Stroke (NINDS) reports that in the United States, diseases of the peripheral nerve affect an estimated number of 20 million people.[1] Strategies for surgical repair of PNIs depend largely on the size of the nerve defect. Direct tension-free neurorrhaphy is preferred for defects less than 5 mm in length,[2] and larger defects are generally treated with autologous nerve grafts, which is the current "gold standard" approach. While autografts have clear advantages in terms of biocompatibility, they also feature several shortcomings, including the need for a second surgery, donor site morbidity, limited tissue availability, size and shape mismatch, and possible formation of painful neuroma.[3,4] These shortcomings have called for the investigation and development of novel therapies, including artificial nerve scaffolds, which hold great promise.

An ideal scaffold needs to feature a combination of optimal material, size, architecture, and surface properties to be fully efficacious in peripheral nerve regeneration. These features allow the formation of a new extracellular matrix consisting of blood vessels, fibroblasts, and Schwann cells, which collectively create favorable circumstances for nerve regeneration to occur.[5]

Biological and synthetic nerve guidance channels, or simply nerve guide conduits (NGC), are a common type of neural scaffold. These devices are tubular structures designed to bridge nerve gaps, protect the injured nerve and prevent scarring, accumulate neurotropic and neurotrophic factors locally, and mechanically guide regenerating axons.[5,6] Various nerve conduits have been approved for clinical use; however, their ability to bridge larger nerve gaps has been largely suboptimal and questionable. Fortunately, over the past few decades, significant advances in neural scaffold technology have been made towards creating biomimetic environments for regenerating axons through the development of improved materials and structures that better reflect natural anatomy and physiology. This review summarizes the current clinical usage and knowledge of neural scaffolds, and highlights new biomimetic technologies in this field. Key aspects of biomimetic neural scaffolds including material, fabrication technique, architecture, and surface properties will be highlighted, and future opportunities for integrating scaffold technologies with other regeneration therapies such as growth factor supplementation and stem cell transplantation will also be discussed.

2. Commercially available neural scaffold technologies

Neural scaffolds are widely used in clinical settings. To date, various bio-absorbable nerve tubes have been approved by the US Food and Drug Administration (FDA) for human use. These devices include: (1) those based on type I collagen: *NeuraGen*™ *(K011168, 2001), NeuroMatrix*™ *(K012814, 2001), and NeuraWrap*™ *(K041620, 2004)*; (2) those based on poly(glycolic acid) (PGA): *Neurotube*™ *(K983007, 1999)*; and (3) those based on poly(D,L-lactide-*co*-e-caprolactone) (PLCL): *Neurolac*™ *(K050573, 2005; K112267, 2011), NeuroMend*™ *(K060952, 2006)*.

For collagen-based nerve tubes, the first commercially available device was *NeuraGen*™ from Integra Lifesciences, Princeton, NJ, which was approved by the FDA in 2001 (K011168). The *NeuraGen*™ device is available in various inner diameters (1.5–7 mm) and two different lengths (2 and 3 cm). Mechanical strength, permeability, and controlled bioabsorption are achieved through maintaining the fibrillar structure of collagen in the fabrication process. Compared with more rigid materials (such as silicone), *NeuraGen*™ exhibited no compression neuropathy,[7] achieving similar efficacy to autografts. [8,9] In terms of bioabsorbability, *NeuraGen*™ is expected to remain structurally intact until up to 4 years post-implantation.[6] After NeuraGen™ was introduced, Collagen Matrix Inc. (Franklin Lakes, NJ) also developed collagen nerve protectant wraps, namely *NeuroMatrix*™; however, to our knowledge, it has not been evaluated in published animal or clinical studies. *NeuraWrap*™ is another available collagen-based device which mimics nerve tubes with adjustable inner diameters ranging from 3 to 10 mm and varying lengths. It remains unclear whether *NeuraWrap*™ is comparable to traditional fixed-size nerve tubes in terms of efficacy.

For PGA based tubes, *Neurotube*™ is a commonly used nerve tube with an internal diameter of 2.3 mm and a length of 4 cm (or as an alternative 4 or 8 mm for internal diameter and 2 cm for length) and is indicated for the repair of peripheral nerve discontinuities not exceeding 3 cm. Clinically, *Neurotube*™ shows promising statistical trends for the repair of short gaps. For digital nerve defects not exceeding 3 cm, treatment with *Neurotube*™ resulted in a higher percentage of patients with “excellent recovery.” *Neurotube*™ has also been shown to mediate recovery following median nerve reconstruction and regeneration of cranial motor nerves.[10,11] For PLCL based technologies, several reports have investigated the applicability of *Neurolac*™ conduits in preclinical settings and demonstrated favorable results in nerve regeneration in the sciatic rat model.[12–14] Secer and colleagues[15] evaluated 455 patients with ulnar nerve injuries and the efficacy of *Neurolac*™. While the conduit showed promising results, more recent data have identified adverse events such as swelling and automutilation, raising concerns about biocompatibility and degradation rate.[16,17] Additional issues including rigidity and patient complications were also reported, resulting in physicians discontinuing the use of *Neurolac*™.[18]

Among *NeuraGen*™, *Neurotube*™, and *Neurolac*™, *Neurotube*™ has been reported to exhibit the poorest results in a head-to-head preclinical comparison.[19] Functional motor recovery in eighty rats with 10 mm sciatic nerve injury was evaluated, and the recovery rate in animals treated with *Neurotube*™ was a mere 15% for compound muscle action

potentials (CMAPs) and 29% for muscle force. Furthermore, while *Neurolac*™ and *NeuraGen*™ both maintained structural stability 12 weeks after implantation, *Neurotube*™ completely collapsed. In another study, *Neurotube*™, *NeuraGen*™, and autografts were compared in the setting of repairing 10 mm rat sciatic nerve gaps.[20] The results showed that *Neurotube*™ conduits led to significantly less organized and less dense axon regeneration compared to *NeuraGen*™ and nerve grafts. Interestingly, conduits fabricated with *Neurolac*™ were shown to be superior to both *Neurotube*™ and *NeuraGen*™,[19] leading to slower degradation and improved maintenance of fluid impermeability.[21] However, due to its rigidity, *Neurolac*™ tubes can potentially complicate surgical fixation and lead to mechanical trauma, fistula formation, extrusion, and inflammatory reaction.[22–24]

Various newer scaffolds have also been recently approved for clinical use. *NeuraGen*™ *3D (K130557, 2014)* is another collagen conduit fabricated with bovine Type I collagen conduit featuring a porous inner hydrogel matrix of collagen and glycosaminoglycan (chondroitin-6-sulfate). *NeuroFlex*™ *(K131541, 2014)* is a newer iteration of the PGA-based device, and *Nerbridge*™ *(K152967, 2016)* from Toyobo Co., Ltd is another PGA based tube, which features a porous collagen filling and properties such as flexibility, resorbability, and semipermeability. Reinforced flexible collagen nerve cuff *(K170656, 2017), Cova*™ *ORTHO-NERVE (K103081, 2012), Neurocap*™ *(K152648, 2016)*, and *Reaxon*™ *Plus (K143711, 2015)* are some of the other neural tubes that are currently commercially available. While each of these novel scaffolds features various differences from their predecessors, thorough preclinical and clinical data are not yet available in the published literature.

Despite the FDA approval of various scaffold technologies, data regarding each device are difficult to compare due to the lack of standardization of pre-clinical models and evaluation methods. Each device has shown variable efficacy in different settings, so the advantages and disadvantages of each conduit must be carefully evaluated in light of varying disease conditions (*e.g.* anatomical location, size of lesion *etc.*), evaluation methods, and patient populations.

Overall, although clinical use of nerve conduits has been extensive, many areas for improvement remain. Optimal peripheral nerve regeneration, particularly for large nerve gaps, is far from realization. Fortunately, many novel biomimetic technologies have shown exceptional potential and currently await further investigation and eventual clinical translation.

3. Peripheral nerve regeneration environment

To optimize peripheral nerve regeneration, neural scaffolds must possess properties that reflect the anatomy of peripheral nerves. A single peripheral nerve contains nerve fibers (axons encased by Schwann cells), which are surrounded by connective tissues (endoneurium, perineurium, and epineurium), capillaries, and vessels. The endoneurium holds axons and Schwann cells together, forming an endoneurial tube. Endoneurial tubes are surrounded by the perineurium, which has connective tissue made of type I and III collagen fibril and elastic fibers interspersed between 15 layers of flat perineurial cells,[25,26] with each

cell layer having nearly complete basal lamina. Like the endoneurium, the innermost basal lamina of the perineurium is made of laminin, fibronectin, and heparan sulfate proteoglycan, with the innermost cell layer linked *via* tight junctions to form a diffusion barrier that regulates the endoneurial environment. Altogether, the endoneurial tubes, blood vessels, and perineurium form nerve fascicles, with thicknesses ranging from 1.3 to 100 μm.[27]

The current knowledge of the anatomy of peripheral nerve tissue provides the foundation on which biomimetic neural scaffolds can be developed, featuring not only a strong outer wall emulating the epineurium to generally guide nerve regeneration, but also fibrous microtubes with biomimetic extracellular matrix (ECM) components (such as collagen, laminin, and fibronectin)[28] that run parallel to the axis of the peripheral nerve emulating the properties of the perineurium. Together, these biomimetic features can foster a supportive milieu for cell survival and development similar to that of normal physiology and anatomy, synergistically promoting tissue morphogenesis, differentiation, and homeostasis.[29] Thus, the field of biomimetic neural scaffold engineering attempts to successfully reproduce the natural and physiological peripheral nerve environment, and has provided new insights and promise to better facilitate nerve regeneration after injury.[30–32] A visual illustration of key anatomical components of peripheral nerves is depicted in Fig. 1.

4. Material

The first step in developing a nerve tube is selecting the material, which is critical for controlling nutrient exchange and biodegradability.[33] There are many factors that impact the efficacy of nerve conduits, including thickness, porosity, and flexibility. Studies have shown that the conduit thickness is closely related to neuroma formation,[34] and that thicknesses greater than 0.81 mm attenuate axonal outgrowth.[35] The association between increased conduit wall thickness and decreased nerve regeneration is likely mediated by decreased nutrient diffusion and wall porosity.[36] *In vitro*, it has been shown using a material degradation and diffusion study that the optimal conditions for peripheral nerve repair are achieved with a conduit wall thickness of 0.6 mm, a porosity of 80%, and a pore size range of ~10–40 μm.[37] In a rodent model, it has also been shown that biodegradable nerve guides optimally aid nerve regeneration when featuring an internal diameter of 1.5 mm and a wall thickness of 0.3 mm.[5] Mechanical properties such as flexibility are also critical, especially for repairing larger nerve gaps, as nerve stumps may need to be bridged across joints.[38] Thus, nerve conduits should feature both sufficient pliability to traverse joints and rigidity to prevent collapse after implantation.

Material selection is another key component of nerve conduits, and can either be biological (autogenous or nonautogenous) or synthetic (bio-absorbable or non-absorbable).[39] In general, there are three main types of materials: (1) biological conduits such as vein and artery grafts; (2) natural polymers such as chitosan, silk fibroin, collagen, chitin and gelatin; and (3) synthetic scaffolds including silicone, polyglycolic acid, poly L-lactic acid, poly-3-hydroxybutyrate, and polytetrafluoroethylene. While biological materials are intuitively superior (such as supporting cell viability, proliferation, intercellular communication, and growth factor elution), there are many limitations. Autogenous materials, which include arteries, veins, muscle, tendon, and epineural sheath, are potentially efficacious as shown by

some experimental use and small case studies; however, this class of nerve conduits has not yet demonstrated enough efficacy to make it to the clinic.[5,40,41] Allografts and xenografts are alternatives to autogenous nerve grafts, as they are readily accessible and unlimited in supply. However, transplantation of allografts usually requires immunosuppressive therapy, and the costs are considerably more expensive.

In order to not only limit immunogenic reactions but also preserve native ECM, decellularized allografts (Avance™ Nerve Graft, AxoGen Inc., Alachua, Florida) have become a viable option and have been approved by the FDA. Despite being widely adopted and utilized in the United States and the general belief that decellularized allografts can bridge large gaps (4–6 cm),[42,43] this technology has only been shown to be consistent for the repairing of small diameters (1–2 mm) and short gaps (<3 cm).[24,44–48] Various preclinical animal studies have evaluated the efficacy of processed nerve allografts relative to other technologies, overall showing that processed allografts are superior to collagen conduits, and similar to autografts and isografts.[49,50]

Surgisis™ Nerve Cuff (K031069, 2003)/Nerve Cuff (K132660, 2014)/AxoGuard™ (K162741, 2016) are other FDA-approved devices featuring porcine small intestine submucosa (SIS) extracellular matrix coaptation aid. *In vivo* studies demonstrated that SIS were superior to silicone conduits, with animals treated with the SIS technology exhibiting superior histological findings and EMG-response (for distal motor latency and amplitude).[51] Further evaluation of SIS *vs.* other conduit technologies awaits controlled clinical trials.

Biological polymers are also highly biocompatible, and are also efficacious for enhancing cell proliferation and nerve regeneration, making them great candidates for nerve conduits.[52] Synthetic nonabsorbable nerve conduits were the first to make it to the clinic, with the first generation using silicone and expanded polytetrafluoroethylene materials (ePTFE, Gore-Tex; W. L. Gore & Associates, Inc., Newark, Delaware). In 1994, Lundborg *et al.*[53] first conducted a prospective randomized trial using silicone tubes, where median nerve gaps of 3 to 5 mm were repaired. Excellent motor recovery was exhibited in the thenar muscles, and sensation nearly recovered to normal levels. Various case series reports have shown that the efficacy of nerve conduits is directly related to the length of nerve gaps, with larger gaps leading to inferior results.[54–56] In addition to its limitations for repairing larger nerve gaps, since biological polymers are non-resorbable, they can often cause compression syndrome with chronic implantation and require an additional surgery to remove the conduit.[57] Other FDA-approved nonresorbable nerve conduits include Salubridge™ (K002098, 2000) and SaluTunnel™ (K100382, 2010). Both are made with PVA hydrogel, and neither devices are validated with published pre-clinical and clinical studies. Overall, clinical utilization of non-absorbable conduits has declined with the advent of absorbable synthetic grafts.

Another possible material that can be used to repair peripheral nerve injuries are xenografts, namely nerve tissue harvested from donor animals of a different species from the recipient. Transplanting xenografts, or xenotransplantation, is a popular subject under active investigation worldwide. With the ever-increasing need for transplantation combined with the shortage of available organs, xenotransplantation has been proposed as a solution to the clinical supply–demand unbalance. [58,59] Replacing damaged human organs using

xenografts, commonly from domestic pigs, can provide a non-toxic and effective solution. Xenotransplantation of peripheral nerves may also be a promising solution for reconstructing large peripheral nerve injuries.[60] While various organs have been proposed to be good candidates for xenotransplantation (such as pancreatic islets, kidneys, heart and liver),[61–63] high immunological rejection due to cross species incompatibility remains a major challenge.[64]

Various attempts have been made at limiting immunological rejection after xenotransplantation. One preclinical study investigated the viability of peripheral nerve xenotransplantation from rats to mice, and demonstrated that brain-derived neurotrophic factor (BDNF) treatment inhibited the immune rejection of peripheral nerve xenotransplantation.[65] Recently, genetically modified animals have been shown to hold great promise to overcome this major immunological obstacle. These modifications are achieved using site-specific nucleases which include zinc finger nucleases, transcription activator-like effector nucleases, and the CRISPR/Cas system, and are administered *via* pronuclear and cytoplasmic microinjection, somatic cell nuclear transfer, and viral transduction of DNA.[66] Genetic modification of donor animals combined with other interventions such as immunosuppressive drugs can further facilitate the successful application of xenotransplantation.[67] Overall, xenotransplantation of nerve tissue harvested from genetically modified donors may be an effective strategy for peripheral nerve repair; however, there are currently very few studies in this area. Furthermore, as peripheral nerve injuries are not life-threatening, genetic modification should be considered with costs in mind, including the potential need for long-term monitoring. Thus, xenotransplantation for peripheral nerve repair remains a work in progress awaiting further investigation.

5. Architecture

The structure of neural scaffolds is extremely important for the efficacy of neural scaffolds and has significantly advanced in recent years. Neural scaffolds now feature greatly improved structural components that reflect the natural composition, shape, and mechanical properties of peripheral nerves, thus better support nerve regeneration. While a simple hollow tube may be sufficient to support nerve regeneration over short distances, research has focused on the development of biomimetic designs such as NGCs with multichannel and physical lumen fillers to improve outcomes following the repair of larger nerve gaps.

5.1 Single hollow lumen NGCs

Historically, single hollow lumen NGCs were the first neural scaffolds to be used. Hollow cylindrical tubes or porous foam rods were used due to their simplicity of fabrication,[68] and were first seen as early as in 1879.[69] Despite the widespread availability of single hollow lumen NGCs in the clinics, results have been inconsistent and largely poor, especially for larger nerve gaps. The limitations of single hollow lumen NGCs for regenerating larger nerve gaps may be due to the inappropriate dispersion of regenerating axons, leading to erroneous target innervation or polyinnervation of multiple targets.[70] Despite good cell attachment and growth, hollow lumens induced *in vitro* shrinkage of regenerated axons.[71] Furthermore, the stiffness of the hollow lumen NGCs was insufficient to resist the

mechanical strain from the surrounding tissue when repairing rat sciatic nerves.[19] Wangensteen and Kalliainen demonstrated that sensory recovery was only achieved in 35–45% of 96 patients as measured by 2-point discrimination, Semmes-Weinstein, and EMG testing.[72] Thomsen *et al.* further demonstrated that among 10 clinical cases, only 50% patients had good outcomes as measured by a variety of methods (2-point discrimination, Semmes-Weinstein, Quick-Dash outcome survey, Cold Intolerance Symptom Severity, and pain recurrence testing).[73] Cumulatively, these disappointing results highlight the limitations of hollow lumen conduits, prompting further development of NGCs with more advanced structural designs.

5.2 Multichannel NGCs

Efforts were made to directly mimic the architecture of nerve fascicles by incorporating microtubes inside the lumen of nerve scaffolds to create channels that will guide the regeneration of individual nerve fascicles. The number of microtubes or channels was predefined to control target reinnervation, with a higher number of channels (2 to 4 channels) reportedly being superior to single channels for nerve regeneration evaluated by simultaneous retrograde tracing.[74] In addition to mechanically guiding nerve fascicles, multiple channels or microtubes also provide an increased surface area for cell attachment and growth factor release, leading to increased facilitation of nerve regeneration. Multichannel NGCs have been shown to promote Schwann cell proliferation *in vitro*,[75] and PLGA conduits with longitudinally organized channels provide favorable regeneration environments in a rat model as shown by an increased percentage of open conduit areas occupied by axon cross sections.[76] However, with their increased complexity of structure, multichannel NGCs may negatively impact critical aspects of NGCs such as permeability and mechanical flexibility.[77] Thus, further improvements and investigations are still needed before considering multichannel NGCs for clinical translation.

5.3 NGCs with physical lumen fillers

To overcome the limitations of single hollow lumen NGCs and multichannel NGCs, researchers have also attempted to introduce material inside the lumen to facilitate nerve regeneration. Normally, when nerve injuries are small, a fibrin cable forms across the injury, allowing for the formation of Bands of Bungner and infiltration of Schwann cells.[78] Sprouting axons then follow the Band of Bungner across the nerve gap and reinnervate their targets distal to the injury. However, when nerve injuries are large, Bands of Bungner fail to form, which significantly compromises regeneration. So far, many fillers (*e.g.* fibers, filaments, gels, and sponges) have been incorporated into conduit lumens to promote cell attachment, proliferation, migration, and regeneration.

The first attempt at incorporating lumen fillers was made by Lundborg *et al.*, where eight polyamide filaments were longitudinally oriented in a 15 mm conduit tube and implanted in rats.[79] Results showed that these fillers were superior to hollow lumens, and since this pioneering work, considerable efforts have made to develop physical lumen fillers. Initially, fiber bundles parallel to the axis of the axon were introduced in the NGC lumen to enhance the guidance of regenerating axons and have been shown to be superior to single hollow lumen NGCs in terms of neurite extension and sodium channel expression in an *in vitro*

model.[80] In rat sciatic models, Ngo *et al.* found that inserting a high density of poly(L-lactic acid) fibers into NGCs allows for significantly more growth (2-fold increase) of myelinated axons compared with empty conduits, [81] and Huang *et al.* showed that inserting a spider-silk-like fiber leads to an 81% increase in axon regeneration compared with the control.[82]

Fillings with intrinsic micro/nanostructures and ECM proteins can augment the specification of neuronal polarity, axon guidance, synaptogenesis, and electric transductions.[83] These fillings can come in a gel-like form or three-dimensional sponges, making them easy to introduce into the lumen of NGCs. The density of the gel or sponge is a critical factor that needs to be optimized since an overly dense matrix may hinder cell motility and axon growth as well as consume the available space in the lumen for nerve regeneration. In dog peroneal nerves, a PGA/collagen NGC featuring an intraluminal collagen sponge, successfully bridged an 80 mm long defect.[84,85] This device was also efficacious in humans, successfully repairing 11 mm and 28 mm facial nerve defects, and 20 mm digital nerve defects.[86,87] Furthermore, Gu *et al.* also developed a chitosan NGC with longitudinal PGA filaments in the interior, which achieved functional nerve recovery after repairing a 35 mm median nerve gap in human subjects.[88,89]

6. Scaffold surface modifications

The interactions between the cell and nerve conduits (cell–ECM, cell–cell) are also key factors that influence many regenerative processes, such as cell differentiation, survival, and migration. Thus, the scaffold surface is a key interfacing component of NGCs that can be manipulated and optimized to best reflect advantageous biological conditions for nerve regeneration. Chemical and biological modifications of nerve tubes hold particularly great promise of improving,[90] as many currently used biomaterials are not optimized for cell adhesion and subsequent proliferation.[91] Many studies have investigated the effect of nerve tube surface properties on nerve regeneration.

6.1 Basic modifications

Various methods have been used to modify scaffold surfaces with biomolecules, for example, physical absorption[92] and covalent conjugation.[93] Chemical modifications are also used to incorporate biomolecules, including wet-chemical methods (such as hydrolysis and aminolysis),[94] ozone treatment,[95] UVtreatment and photo-grafting,[96] self-assembly,[97] and plasma treatment.[92] Physical properties such as wettability can be modified *via* chemistry, which can also be used to enable additional binding of ECM proteins and biomimetic (discussed below) to the scaffold surface.

6.2 ECM proteins

ECM proteins can be used to coat the luminal surface of neural scaffolds, which can potentially improve cell adhesion and proliferation as well as neurite outgrowth.[98,99] Various studies have investigated the efficacy of introducing ECM proteins as NGC luminal wall coating. Laminin is the most frequently used ECM protein for the surface modification of neural scaffolds, and has ultimately led to improved Schwann cell viability in *in vitro* assays and nerve regeneration *in vivo* using a 10–12 mm rat sciatic gap injury model.[100]

Though less potent than laminin, other ECM molecules such as fibronectin and collagen can also improve SC adhesion and proliferation *in vivo*.[101]

6.3 Peptide mimetics

One drawback of ECM molecules is that they are generally large and difficult to synthesize, [102] prompting the development of alternatives such as the use of smaller molecules like short chain protein peptide mimetics. These molecules are selective in cell binding and can be incorporated onto neural scaffold surfaces at higher densities. Specific amino acid sequences, such as IKVAV (Ile-Lys-Val-Ala-Val),[103] YIGSR (Tyr- Ile-Gly-Ser-Arg),[104] and RGD (Arg-Gly-Asp),[105,106] have been shown to enhance the adhesion and proliferation of neural cells, and many more have been investigated for use in peripheral nerve regeneration. In a rat 15 mm nerve gap model, Itoh *et al.* demonstrated that laminin and YIGSR peptides do not lead to different outcomes, and both improve nerve regeneration. [107] Furthermore, RGD functionalized conduits are also effective in repairing 10 mm sciatic nerve gaps, yielding results comparable to that of autografts in terms of muscle reinnervation and functional recovery.[108] Schwann cell adhesion can also be augmented by RGD functionalization.[109] However, while SC growth is enhanced with RGD at lower doses, fibronectin signaling and overall nerve regeneration may be negatively affected at higher doses.[110]

7. Fabrication

In order to incorporate the advances in materials, architecture, and surface modifications into neural scaffolds, powerful fabrication techniques must be employed. Neural scaffolds are conventionally made using rolling of a mesh.[111] Traditional fabrication techniques include injection molding,[112] mandrel coating or dip coating,[113] porogen-leaching,[114] freeze-drying, [68] solvent- or thermally induced phase separation,[115] and computer- aided methods such as fused deposition and soft lithography. [116] Among these techniques, injection molding is the most popular as it can be used for most polymer materials when constructing a nerve conduit. Mandrel/dip coating is also a powerful method, and can produce nerve conduits with improved control on thickness and homogeneity. Particulate leaching, gas foaming, and phase-separation techniques can be used to construct permeable nerve tubes. Finally, freeze-drying and lyophilization are used to dehydrate and demold natural polymers, and have been used to construct oriented porous materials in nerve tubes by unidirectional freezing followed by freeze-drying.

For the construction of submicron-sized fibers, electro-spinning is a particularly powerful technique. Tubular conduits can be prepared by rolling up electrospun fibrous membranes and securing the edges with solvent, glue, or heating.[117] More importantly, electrospun nanofibers are able to provide guidance for nerve regeneration by closely interacting with the growth cone, and also establish fibrous structures that resemble the native ECM of neural tissues.[118] Adhesion properties, along with neural precursor viability and differentiation, have been shown to be heavily influenced by fiber diameter and orientation.[119] Overall, longitudinally aligned nanofibers and nanogrooves improve peripheral nerve generation, and have been shown to be beneficial for histological outcomes, nerve conductivity, muscle

atrophy, and functional performance. [120] More specifically, axially aligned fibers have been shown to be superior to circumferentially or randomly aligned fibers in NGC repair of 15 mm nerve defects.[121] Furthermore, cell orientation, migration, and differentiation have been shown to be markedly improved when using highly aligned nanofiber scaffolds.[122–124] Overall, aligned nanofibers not only provide physical guidance,[125] but also foster accelerated regeneration by supporting axonal path finding during regrowth.[126] 3D cylindrical electrospun scaffolds with nano-rough sheaths and aligned cores mediate nerve cell attachment, penetration into the inner structure of the scaffold, and orientation along the core fiber direction.[127] This design mimics the native nerve's fascicular architecture and ECM, and guides neurons and Schwann cell proliferation through its complex 3D construct. Electrospun nanofibrous conduits can also be combined with physical and biochemical cues. This technology features a bilayered design where the inner lumen consists of longitudinally aligned fibers while the outer surface is made of randomly oriented fibers, mimicking the natural hierarchy of native peripheral nerves.[122] When further functionalized with laminin and growth factor, this device showed promising results, surpassing autografts in terms of muscle reinnervation.[101]

Due to limitations in the manufacturing processes, fabricating conduits with uniform channel diameter and nanodesigns is very challenging. Recently, 3D printing has emerged as a novel technique capable of fabricating scaffolds with specific shapes and dimensions tailor-made to patient needs, and can precisely produce complex and arbitrary three-dimensional objects. [128] 3D printing technology offers an integrated approach for combining synthetic materials, biomaterial, growth factors along with shapes/sizes specific to device recipients determined by medical imaging.[128] Several recent studies have explored constructing neural scaffolds with 3D printing technology. Using 3D printing technology, Johnson *et al.* developed bifurcation nerve scaffolds with imbedded growth factor gradients for the first time, and demonstrated its therapeutic effect in a rat nerve regeneration model.[129] Our approach loaded path specific neurotrophic factors (NGF for axons and GDNF for Schwann cells) to promote selective regeneration of peripheral nerves, and *in vivo* results demonstrated that functional recovery was improved with these novel devices.[129] Yurie *et al.* further confirmed in rats that 3D printed conduits promoted nerve regeneration.[130] Many reports have shown that 3D printing technology provides an exciting new fabrication technique that may be able to fulfill clinical needs for complicated and patient-specific artificial nerve grafts which cannot be satisfied by conventional fabrication techniques. Despite exciting preclinical results, 3D printing technology is still relatively new and largely unvalidated clinically. Currently, this technology is limited by the lack of available biomimetic material "inks", and suboptimal nano-resolution of the printed devices. Thus, optimization of 3D printing fabrication of NGCs requires the novel development of biomimetic materials and further improvements in 3D printer resolution.

8. Synergy with other peripheral nerve regeneration therapies

While neural scaffolds are the cornerstone technologies for repairing peripheral nerve gap injuries, they have only been shown to be efficacious for small lesions (<10 mm in rats or <30 mm in primates). For larger injuries, scaffolds have been largely unable to achieve adequate regenerative effects when used alone. In addition to the various aforementioned

strategies to augment the efficacy of nerve tubes, non-scaffold modifications such as growth factor supplementation and stem cell transplantation can also be incorporated into advanced scaffold technologies to further optimize peripheral nerve repair.[40,41]

8.1 Growth factor supplementation

Growth factors are widely known to be beneficial to nerve regeneration, significantly improving the efficacy of peripheral nerve injury treatment as shown by a meta-analysis of twenty clinical studies.[131] While these substances are excreted by endogenous cells during regeneration and biomimetic neural scaffolds can augment endogenous expression, supplementing the nerve regeneration environment with exogenous growth factors can further improve the outcomes. Commonly studied growth factors include brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), vascular endothelial growth factor (VEGF), and neurotrophin-3 (NT-3), which are known to have specific affinities with specific ECM proteins.[132] Many of the novel advances in biomimetic neural scaffold technology have incorporated growth factor supplementation and achieved promising results. For example, specific growth factors can be integrated into various locations of 3D printed neural scaffolds for bifurcating nerve gaps, allowing for possibly better innervation specificity of the motor and sensory neurons.[129] Growth factors can also be encased in physical lumen fillings using hydrogel-forming collagen, alginate, heparin, and heparin sulfate, which can sustain the efficacy and prevent degradation of growth factors.

Since dense lumens may hinder nerve growth (as discussed previously), growth factors can be attached to scaffold walls to reduce clutter. Growth factors integrated into the scaffold material can prolong localized drug availability over time.[133–136] Incorporation of various growth factors (such as NGF,[133,134] CNTF,[136] GDNF,[135] and VEGF[134]) into the luminal wall has been studied extensively. Overall, integrating growth factors into the conduit walls may be a superior strategy compared to luminal fillings as the lumen can remain open for axonal growth. However, as mentioned previously, there are shortcomings of an open lumen design, such as a lack of structural support for cellular axonal growth. Using functionalized materials for neural scaffolds in conjunction with growth factors may have synergistic effects and significantly improve outcome; however, the optimal method of incorporation (wall *vs.* lumen) awaits thorough investigation.

8.2 Stem cell transplantation

Stem cell transplantation is another widely studied strategy for peripheral nerve regeneration as we recently reviewed,[40] and is inherently advantageous compared to growth factor or protein supplementation due to stem cells' ability to respond to the needs of regenerating tissue and secrete appropriate pro-regenerative factors. NGCs are critical to stem cell transplantation, and can house the injected stem cells to ensure optimal local concentration at the site of desired repair. Furthermore, stem cells can be seeded within hydrogels or into channels of multichannel NGCs. Overall, stem cell transplantation into NGCs has shown positive results.

Various pre-clinical animal studies have shown convincing evidence that stem cell transplantation can serve as adjunct therapy to traditional methods of peripheral nerve repair.[40,137,138] However, while stem cell transplantation seems to show great potential, current technologies are yet to identify optimized conditions for clinical use in terms of both efficacy and safety. To achieve clinical efficacy, graft survival must be adequate. Currently, the survival of the transplanted stem and precursor cells is widely variable (between 0.5 and 38%) depending on the cell type, method of delivery, and evaluation time point. Thus, in order to fully realize the potential of stem cell transplantation in peripheral nerve repair, determination of the optimal cell type, cell number, and delivery method must be made. Bhangra *et al.* summarizes the current preclinical literature on stem cell transplantation in NGCs for peripheral nerve repair.[139] While many challenges remain in optimizing stem cell transplantation, the critical need for new therapy in neurological disorders has fueled numerous clinical trials (ClinicalTrials.gov identifier NCT01739023 and NCT01909154), especially in diseases involving the central nervous system. Currently, however, there are no reported clinical data regarding cell-based therapy for diseases of the PNS. Overall, cell therapy in peripheral nerve regeneration awaits further strengthening of preclinical evidence before progressing to clinical trials and translation.

8.3 Metabolic glycoengineering and modification of cell surfaces

In addition to modifying the characteristics of neural scaffolds to interact better with cells, cells can also be modified to improve the interactions with neural scaffolds and ultimately lead to better regeneration. While ECM glycoproteins are involved in cellular adhesion, glycans expressed on cell surfaces are also critical. Similar to ECM glycoproteins, cell surface glycans have a wide variety of functions, and modifying them can alter cell adhesion, cell differentiation, and both intra and intercellular signaling, and ultimately improve cell outcomes in nerve regeneration.[124]

Reagents for glycan engineering are commercially available, making it easy for researchers to implement them. One efficient way to modify cell surface glycans is metabolic glycoengineering (MGE),[140,141] which manipulates biosynthetic pathways for oligosaccharides and glycoconjugates by using sugar analogs.[142] For example, functional groups like azide,[143] ketone,[144] and thiol[145] can be attached to cell surfaces *via* sialic acid glycoengineering. This has allowed for the selective decoration of cell-surface polysialic acids,[146] making it possible to attach modified sialic acids for various purposes.[147]

MGE has been shown to have the ability to alter cell adhesion *via* activation of integrins[148] and modifying the levels of cell adhesion molecules[149,150] *via* the introduction of propanolylated sialic acids to the cell surface.[150–152] Modified sialic acids can stimulate axonal growth,[153] induce neuronal differentiation of PC12 cells[145,154,155] and oligodendrocyte progenitor cells.[154] Yarema's group showed that the MGE-induced increase in adhesion improves the attachment of non-adhesive blood cells (the Jurkat line) to surfaces.[124] Increasing cell adhesion also changes cell fate, with modified human embryonic stem cells differentiating when grown on a gold surface.[145]

Carbohydrate based cell surface modification has been studied in animals, including rats[156] and mice,[157] demonstrating improved peripheral nerve regeneration in a mouse model

without apparent toxicity.[158–160] These lines of evidence indicate the feasibility of glycoengineering precursors, and highlight the potential for future development and optimization of neural scaffolds towards clinical translation.

9. Conclusion

Neural scaffolds hold great promise in the field of peripheral nerve regeneration; however, the currently approved options are still far from satisfactory, particularly for larger gap injuries. Various efforts have attempted to improve on the current technology, and better emulation of the anatomy and physiology of peripheral nerve tissue has shown promising results. While autologous grafts remains the gold standard approach, biomimetic technologies in scaffold design have improved the regeneration performance of neural scaffolds. To fully realize the clinical potential of these new technologies, multifunctional devices combining biomimetic material, shape, structure, and surface modifications should be investigated in-depth. Furthermore, new technologies in neural scaffold engineering may have additional synergistic effects with other pro-regenerative strategies such as growth factor supplementation, stem cell transplantation, and cell surface glycoengineering (Fig. 2). Overall, recent advances in biomimetic technology present exciting new possibilities towards the development of artificial neural scaffolds that may surpass the efficacy of the current clinical practice.

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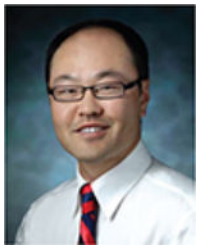
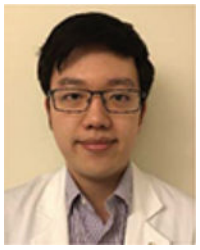
Biographies



Jian Du

Dr Jian Du received his BSc from the Chemical & Biomolecular Engineering Department of Liaoning University in 2000, and his Ph.D. in biomedical engineering in 2005 from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences. After he graduated, Dr Du joined the University of California at Davis as a post-doc researcher in 2008, and worked as a research fellow at Johns Hopkins University (2008–2014) and University of Maryland School of Medicine (2015 to the present). His research interests are to merge three lines of research that have independently shown promise in advancing tissue engineering endeavors: biomaterial scaffolds, nanotechnology, and stem cell research.



Huanwen Chen

Huanwen “Alvin” Chen earned his BA in physics from Washington University in St Louis in 2015 and is now an MD student at the University of Maryland School of Medicine. His research interests include translational models for nerve injury and repair, with a focus on novel therapies for peripheral nerve regeneration. He developed a reliable method to evaluate the gait function in rats after sciatic nerve injury using an automated system, and this work was recently published in the *Journal of Neuroscience Methods*. Alvin is also involved in immunology and epidemiology research at the University of Maryland School of Medicine.

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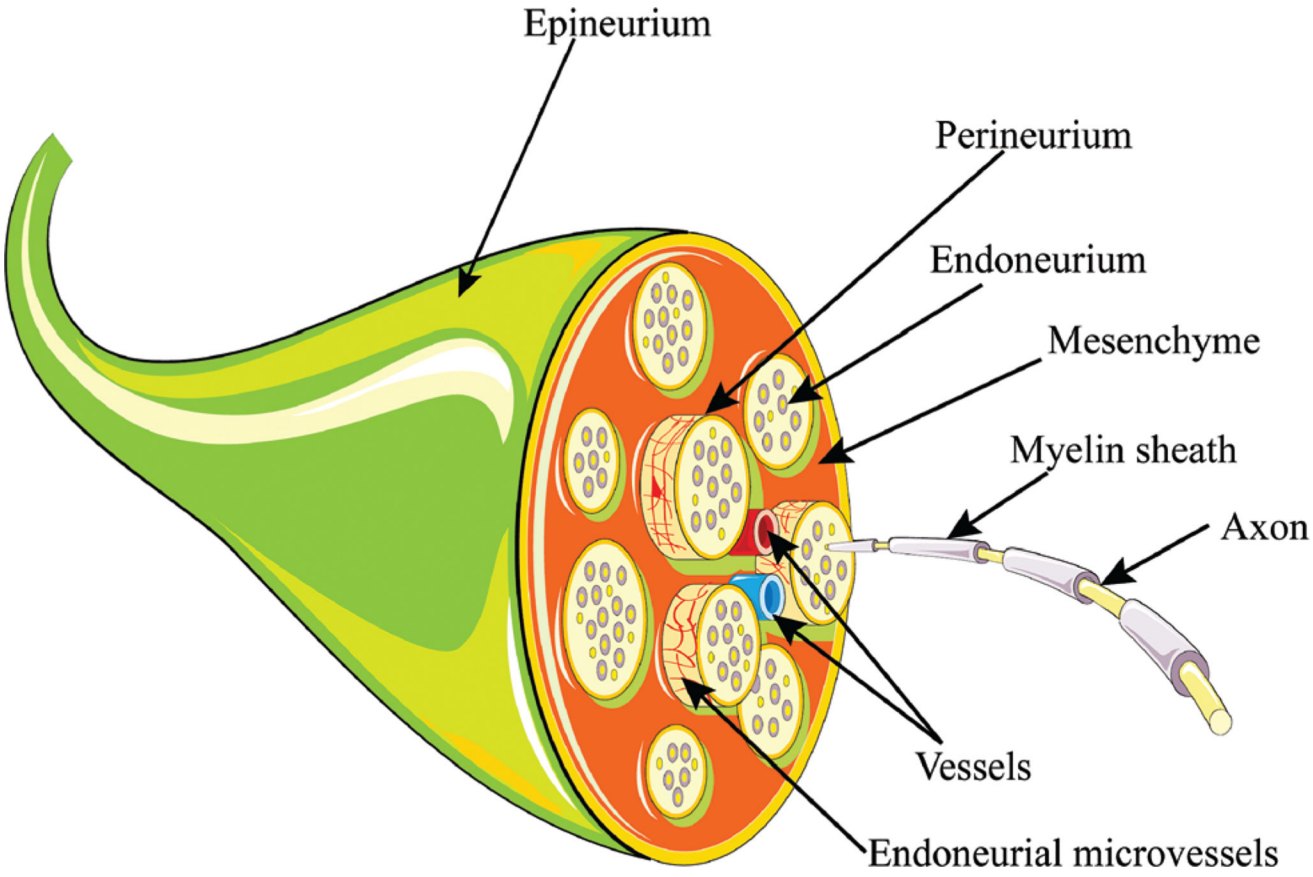

Fig. 1.
Visual representation of key anatomical components of peripheral nerves.

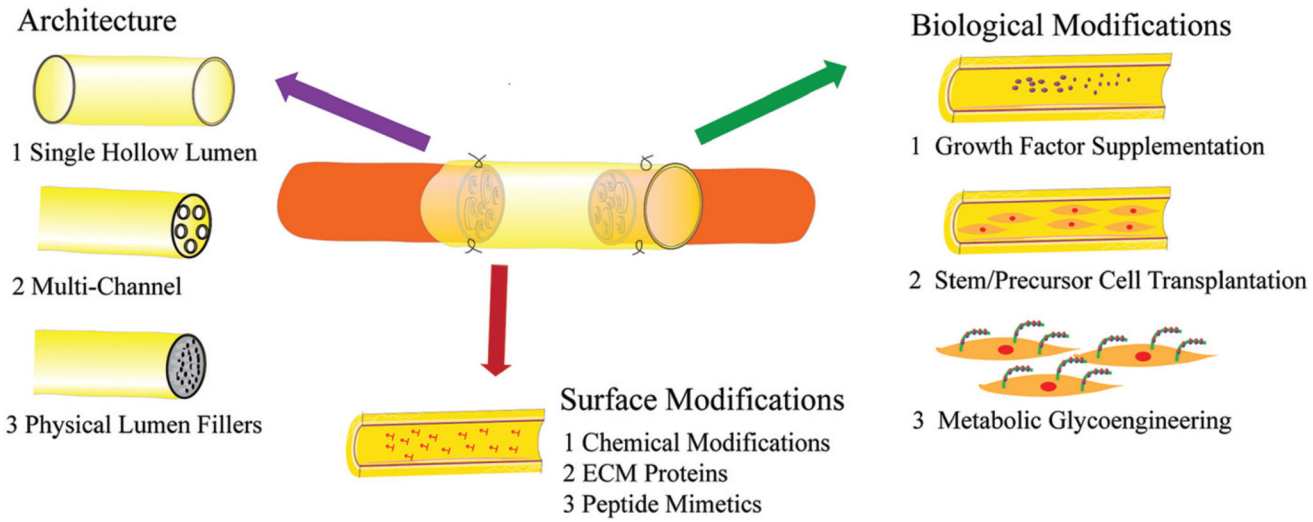

Fig. 2.
Schematic diagram of various biomimetic neural scaffold technologies.