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Advances in Nerve Guidance Conduit-Based Therapeutics for Peripheral Nerve Repair

AUTHOR(S)

William A. Lackington, Alan J. Ryan, Fergal O'Brien

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Advances in nerve guidance conduit-based therapeutics for peripheral nerve repair

Authors

[William A. Lackington](#)^{1,2,3}, [Alan J. Ryan](#)^{1,2,3}, and [Fergal J. O'Brien](#)^{1,2,3*}

¹Tissue Engineering Research Group (TERG), Department of Anatomy, Royal College of Surgeons in Ireland.

²Advanced Materials and BioEngineering Research (AMBER), Trinity College Dublin, Ireland.

³Trinity Centre for BioEngineering (TCBE), Trinity College Dublin, Ireland.

*Phone +353 1 402 2149, E-mail: fjobrien@rcsi.ie

Abstract

Peripheral nerve injuries have high incidence rates, limited treatment options and poor clinical outcomes, rendering a significant socioeconomic burden. For effective peripheral nerve repair, the gap or site of injury must be structurally bridged to promote correct reinnervation and functional regeneration. However, effective repair becomes progressively more difficult with larger gaps. Autologous nerve grafting remains the best clinical option for the repair of large gaps (20-80 mm) despite being associated with numerous limitations including permanent donor site morbidity, a lack of available tissue and the formation of neuromas. To meet the clinical demand of large gap repair and overcome these limitations, tissue engineering has led to the development of nerve guidance conduit-based therapeutics. This review focuses on the advances of nerve guidance conduit-based therapeutics in terms of their structural properties including biomimetic composition, permeability, architecture, and surface modifications. Associated biochemical properties, pertaining to the incorporation of cells and neurotrophic factors, are also reviewed. After reviewing the progress in the field, we conclude by presenting an outlook on their clinical translatability and the next generation of therapeutics.

Keywords

Peripheral nerve repair, nerve guidance conduits, Schwann cells, neurotrophic factors

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1. Introduction

Peripheral nerve injuries (PNI) arising from acute trauma can result in complete loss of motor functionality and muscle paralysis. The radial and peroneal nerves, of the upper and lower limbs respectively, are amongst the most frequently injured nerves^{1,2}. In the US alone, half a million surgical procedures are carried out annually, creating a market for repair estimated to be worth \$1.5 billion dollars³. With a further 300,000 PNI cases reported in Europe every year, it is estimated that over 5 million cases of PNI occur annually worldwide⁴. The focus of this review is on the most severe type of injury referred to as neurotmesis and Grade V according to Seddon⁵ and Sunderland⁶ classification systems respectively, which are characterized by a full transection of the injured nerve, resulting in a gap between nerve stumps that cannot be directly coapted due to the resulting tension in the sutured nerve, or alternatively cannot heal itself if left untreated^{7,8}.

Peripheral nerves respond to injury by attempting to regenerate. Proximally to the site of injury, neurons activate intrinsic signaling pathways that are regulated by transcriptional factors and promote outgrowth^{9,10}. Distally to the site of injury, axons degenerate while Schwann cells undergo de-differentiation into a non-myelinating, pro-regenerative phenotype associated with increased proliferation, migration, secretion of neurotrophic factors, and deposition of basal lamina proteins^{11,12}. Subsequently, Schwann cells begin to migrate across the site of injury in order to promote the formation of bands of Büngner, which are aligned tracks of basal lamina proteins that support the guidance of outgrowing axons^{13,14}. In addition to debris clearance by an elaborate and controlled immune response, this response to injury is referred to as Wallerian degeneration^{15,16}. However, large gaps, particularly those seen clinically (larger than 20 mm), typically hamper this natural response to injury, and

therefore PNI of this calibre require treatment to bridge the gap and support this response to injury.

Autologous nerve grafting is the current clinical gold standard treatment for large gap PNI. The procedure involves donor graft harvest, typically from the sural nerve of the patient, and subsequent transplantation to bridge the site of injury¹⁷. Limitations with autografting include permanent donor site morbidity, and functional loss in the donor 'sacrificial' nerve¹⁸. Another major limitation of autografting is that Schwann cells may undergo necrosis within the graft once transplanted due to limited perfusion from the surrounding vasculature¹⁹. This occurs when the graft is too thick and revascularization cannot reach the center of the graft, or when the graft is too large and surrounding vasculature cannot provide sufficient nutrients and oxygen to the entire graft, resulting in poor functional repair²⁰. Other limitations include the requirement for multiple invasive surgeries, increased risk of infection, and the limited availability of graft tissue for severe cases where large amounts of donor tissue are required. However, under ideal conditions autografts perform quite well in some cases due to their native physical structure that acts as a template for regeneration, and their biochemical environment consisting of both Schwann cells and neurotrophic factors that support repair. The physical structure of autografts is similar to that of the distal nerve stump found after injury, consisting of the connective tissue that surrounds axons in a healthy nerve. However, axons within a graft degenerate and the connective tissues provide a unidirectionally aligned three-dimensional network of extracellular membrane (ECM) proteins and basal lamina that can act as a protective architectural template for the growth of axons and migration of Schwann cells across the site of injury (Fig. 1). Additionally, autografts contain endogenous neurotrophic factors and Schwann cells from the donor site. These Schwann cells have a non-myelinating, pro-regenerative, and neurotrophic factor-producing phenotype since they, like Schwann cells at the distal end of the site of injury, undergo reprogramming in response to injury²¹. However, due to the limitations associated with autografts alternative therapeutics are required to improve the prognosis for patients.

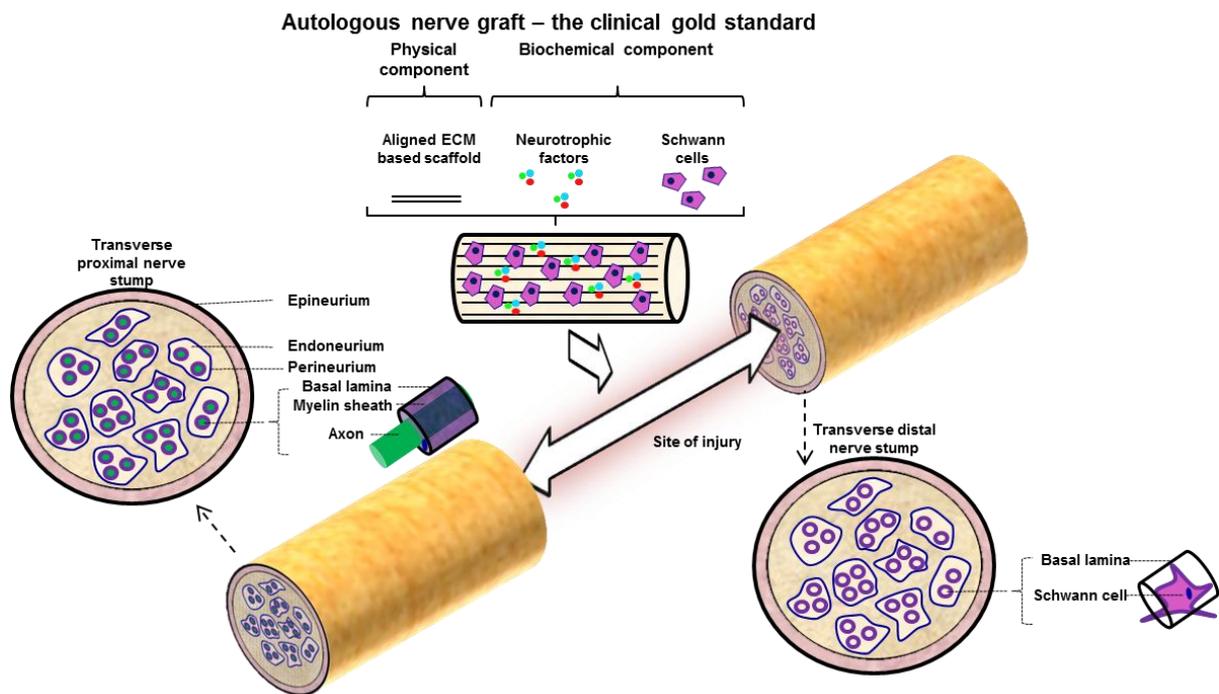


Figure 1. Autografting and considerations for the development of alternative therapeutics
 In ideal circumstances, repair at the site of injury is followed by distal target reinnervation, which results in the return of nerve functionality. The migration of Schwann cells across the site of injury is critical for successful repair. However, this is often met with natural senescence in larger gaps. In autografting, the natural senescence of Schwann cells is alleviated by the presence of autologous Schwann cells in the same de-differentiated state, in addition to the aligned ECM-based connective tissue, and the plethora of neurotrophic factors found within the graft.

Tissue engineering (TE) has emerged as a potential solution to overcome the limitations of autografts. Typical TE strategies focus on the treatment of injuries by implanting natural, synthetic or semisynthetic biomaterial-based nerve guidance conduits (NGCs), which may incorporate biochemical cues such as cells or neurotrophic factors. NGCs are primarily tubular constructs, which may be hollow or filled throughout their luminal space with an additional biomaterial supporting structure. They are designed to bridge injury sites and provide a physical template for the regeneration of peripheral nerves. A number of distinct NGC-based therapeutics are now commercially available^{22,23} but their performance has yet to surpass that of autografts in the treatment of injuries, particularly in the repair of large gaps seen clinically (>20 mm) and therefore, their application is currently restricted to small gap repair only²⁴. Additionally, commercially available tubular constructs have yet to receive regulatory approval to incorporate biochemical components such as cells or neurotrophic factors, which, as this review will illustrate, can significantly enhance their repair capacity.

2. Improved NGC structural composition leads to superior repair

Significant advances have been made developing NGCs with improved structural compositions. The biomaterial composition can vary greatly but a number of observations

can be made to highlight recent advances. The following sections focus on these observations:

2.1 Biomimetic design is a leading approach in the development of NGCs

A biomimetic approach has been used with success in the development of tubular constructs and is defined by one that mimics the natural composition, shape, or mechanical properties of peripheral nerves, its ECM, and its basal lamina²⁵⁻²⁷. Biomaterials used for this purpose include polymers of natural mammalian origin such as collagen from bovine tendon or calf skin²⁸. Non-mammalian sources of collagen such as fish also exist²⁹, however mammalian sources of collagen are predominantly used in research and development of collagen-based scaffolds. In this section, the focus will be on collagen as a biomimetic biomaterial due to its widespread, successful, and continued use in tubular constructs for peripheral nerve repair.

The use of collagen as a biomaterial for PNR began in the 1980s after the problems with first generation non-biodegradable hollow tubular constructs became apparent²⁸. Historically, their biomaterial composition included non-biodegradable silicone, which was associated with long-term complications arising from scar tissue formation and foreign body reaction³⁰. In contrast to silicone, collagen is the most abundant protein found in mammals and plays a significant role in the structural maintenance of ECM in various tissues including peripheral nerve. As such, collagen is biodegradable, biocompatible, and immunologically inert for many applications³¹⁻³³. In peripheral nerves, collagen plays a role in the maintenance of the basement membrane, which is synthesized and regulated by Schwann cells³⁴. The basement membrane of peripheral nerve is a key connective and structural tissue that supports both Schwann cells and neuronal axons. Therefore, collagen can be used to mimic the healthy structural composition of peripheral nerves with the aim of providing a suitable scaffold for migrating Schwann cells, regenerating axons, and *de novo* tissue formation *in vivo*. In this context, collagen can be incorporated into either or both the outer tube wall and the luminal space as a luminal filler component in tubular constructs.

This rationale was demonstrated in 1990 when a 5 mm rat peroneal nerve defect was successfully bridged using a collagen-based NGC³⁵. It was composed of a synthetic outer tube made of polyglycolic acid filled with a biomimetic luminal filler made of collagen. The axonal regeneration was comparable to that of autografts. Conversely in 1997, hollow collagen-based tubular constructs failed to support axonal regeneration across a larger 18 mm gap in the sciatic nerve of rats³⁶. These two early studies suggest that collagen-based tubular constructs can support the repair of injuries but larger gaps present a challenging environment for effective repair.

The mechanical properties of tubular constructs can also be designed to mimic that of healthy nerves. Nerve tissue is typically classified as soft tissue and has a relatively low

stiffness (brain matter <1 kPa, spinal cord 90-230 kPa, peripheral nerve 150-300 kPa)³⁷⁻³⁹. However, an important question arises in whether NGCs should be designed to have mechanical properties that mimic native nerve or to provide the best template scaffold for repair, even if this means the mechanical properties of NGCs differ to those of native nerve. In 2012, Gu *et al.* investigated this by assessing the crosstalk between Schwann cells and their surrounding microenvironment, finding that a matrix stiffness of 7.45 kPa was optimal for Schwann cell biological performance⁴⁰. Similarly, Engler *et al.*, approximated that a matrix stiffness of 1kPa, albeit in 2D collagen-coated polyacrylamide-based substrates, was optimal for neural stem cell differentiation⁴¹. Recently, Rosso *et al.* ratified the importance of substrate properties on the mechanosensitivity of neural cells, finding how neural cell activity is highly dependent on substrate stiffness⁴². These correlating results suggest that a significantly lower stiffness to that of native peripheral nerve supports adequate neural cell activity for repair. However, further studies are required to fully elucidate the influence of environmental mechanical properties on nerve repair and specifically how mechanical cues modulate pro-regenerative neural cell activity.

2.2 Radial permeability is a key mediator of NGC success

Tubular constructs, filled or hollow, may have, depending on their fabrication technique, impermeable, semi-permeable or fully permeable outer tube walls or luminal fillers that control access of nutrients, growth factors, waste products, revascularization, and scar-producing fibroblasts in a radial direction between the site of injury and surrounding microenvironment (Fig. 2). The radial permeability of tubular constructs has increasingly been identified as a key mediator of both hollow and filled NGC success^{43,44}. Fibroblasts can deposit axon repulsive proteoglycans such as chondroitin sulfate and impede nerve regeneration⁴⁵⁻⁴⁷. Based on this, both biochemical and physical strategies have been developed to prevent fibroblasts from deposit scar tissue at the site of injury, and thereby promote regeneration. For instance, the calcium channel blocker, verapamil, has been shown to reduce scar formation *in vitro* by inhibiting fibroblast adhesion, whilst *in vivo* it has been shown to suppress fibroblast secretion of collagen I & III and promote axon growth⁴⁸. Alternatively, fibroblast migration into the site of injury can also be controlled physically by having radial semi-permeability that prevents fibroblast migration into the site of injury but permits revascularization, and the exchange of vital nutrients and growth factors. This has been demonstrated in a study by Oh *et al.* in which a NGC was developed with inner wall nanosize pores (~50 nm) that could limit fibroblast infiltration but permit vital nutrients and growth factor exchange, while the outer surface was designed to have microsize pores (~50 µm) and permit revascularization into the site of injury⁴⁹.

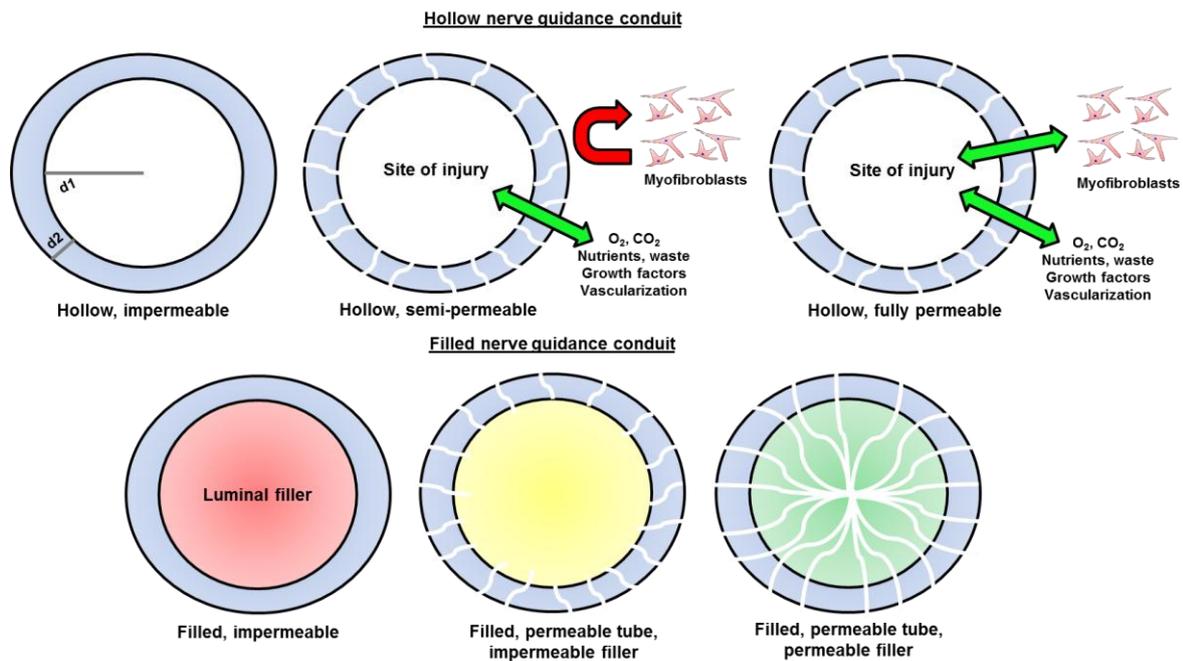


Figure 2. Considerations for radial permeability in NGCs
 Radial permeability of tubular constructs can modulate the inflow of growth factors and nutrients, the outflow of cellular waste products, and revascularization, and therefore directly affects the local regenerative microenvironment. The distance from the tube wall to the center of the site of injury (d_1) and the distance of the tube wall itself (d_2) are important factors that affect radial permeability. Hollow tubular constructs with permeable outer tube walls perform significantly better than with impermeable outer tube walls⁵⁰. Distinct tubular constructs have been made with either fully-permeable or semi-permeable tube walls or luminal fillers, where the latter prevents the entry of myofibroblasts into the site of injury⁴⁷.

Historically, hollow tubular constructs composed of non-permeable silicone were easily surpassed in terms of repair capacity when their outer walls were replaced by permeable silicone walls⁵⁰. To further understand the effects of permeability, in 2005 the role of structural parameters such as wall thickness and porosity were assessed using a tubular construct with both the tube wall and luminal filler composed of collagen⁵¹. This study reported that poor porosity throughout a luminal filler led to inadequate repair due to limited permeability to the surrounding microenvironment. In contrast to this, a tubular construct of similar composition but fabricated using freeze drying, which created a highly porous luminal filler by controlling the rate of ice crystal formation (freezing step) and subsequently sublimation (drying step), led to superior repair due to its increased permeability⁵².

Fully- and semi-permeable structures, either tube walls or luminal fillers, have since been investigated, and conflicting results have been attained. Fully-permeable structures are permeable to both cells and nutrients, and have shown to improve functional recovery over semi-permeable ones, which are only permeable to the diffusion of nutrients, waste products and important growth factors but impermeable to cells⁴⁴. These findings suggest that, contrary to the prevailing dogma, infiltrating fibroblasts may also have a positive effect on nerve repair. The impact of fibroblasts has been investigated on Schwann cells and

interestingly, it has been found that fibroblasts can increase the migratory activity of Schwann cells by the secretion of neuregulin⁵³. Conversely, it has also been shown that semi-permeable structures compared to fully-permeable ones may also improve functional parameters such as nerve conduction velocity by limiting fibroblast access to the site of injury⁴³. Further studies should elucidate the exact effects of fibroblasts on the inhibition or indeed the support of nerve repair. Systems with altered inflow and outflow permeability have also been developed, where the permeability towards the center of the site of injury (inflow) is low but the permeability towards the surrounding microenvironment (outflow) is high. This approach has resulted in enhanced repair compared to uniform inflow and outflow⁵⁴. Collectively, these studies demonstrate a transition in the field from less permeable and hollow tubular constructs with limited success towards filled semi-permeable NGCs with increased success.

2.3 From the macro- to the nanoscale in NGC architectural modifications

The suboptimal performance of hollow tubular constructs, particularly seen across large gap repair, formed the rationale for researchers to investigate the use of filled NGCs whereby the hollow luminal space of tubular constructs is replaced by additional supporting biomaterial in order to enhance their performance. For example, one of the simplest structural modifications is to introduce a hydrogel-based luminal filler, with results showing that hydrogels composed of natural polymers such as collagen and laminin to be particularly effective at enhancing repair⁵⁵⁻⁵⁷. While the addition of hydrogels can provide enhanced repair, microscale architectural guidance has been identified as a key parameter in the regenerative process. It has now become widely accepted that Schwann cell migration and axonal growth is led by the presence of physical support and guidance structures at the cellular level, often referred to as the contact guidance theory⁵⁸. Thus, the presence of microscale alignment in the proximo-distal direction (unidirectionally) within the architecture of tubular constructs can potentially enhance repair at the cellular level by increasing the efficiency of Schwann cell migration and axonal pathfinding^{59,60}. Axonal pathfinding refers to the mechanism by which axons extend in a directed manner by interacting with attractive or repulsive cues that are translated into intracellular retrograde signals by the growth cone, or forefront of axons⁶¹. However, control over the complexity in the inner architecture of tubular constructs has historically been limited by the available bio-fabrication techniques at that time (Fig. 3).

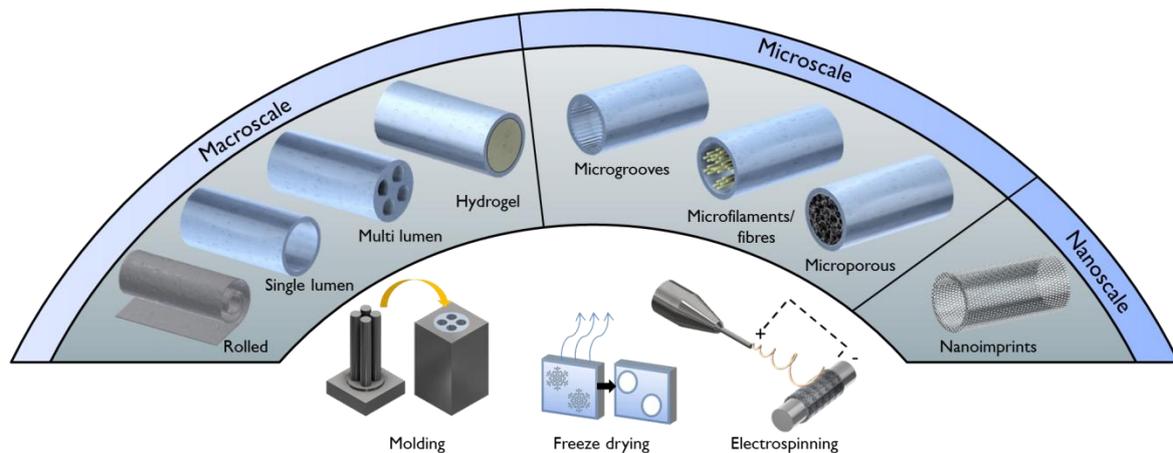


Figure 3. Architectural modifications of tubular constructs and some possible fabrication techniques. Architectural modifications to the traditional design of single lumen tubular constructs can be made at macro-, micro-, and nanoscale levels. Single lumen³⁶, multi lumen⁶², rolled⁵¹, and hydrogel⁵⁷ are examples of tubular constructs with macroscale architectural modifications. Microgrooves⁶³, microfilaments or microfibrils⁶⁴ and microporous⁶⁵ are examples of microscale modifications, while nanoscale modifications include nanoimprints⁶⁶. The scale of architectural modifications is largely dependent on the technology used to fabricate the tubular constructs. For example, molding can be used to fabricate multi lumen NGCs by shaping materials at the macroscale, while more advanced techniques such as nanoimprint lithography⁶⁶ are required to modify tubular constructs with nanometer scale patterns. Freeze drying⁶⁷, which generates pores in the micrometer scale due to the controlled sublimation of ice crystals, and electrospinning⁶⁸, which produces micro-sized fibers by using electric force, are two of the most widely used techniques to modify the microscale architecture of tubular constructs.

2.3.1 Macroscale luminal architecture

Fabrication techniques initially allowed for architectural modifications at the macroscale level. For example, molding of tubular constructs with shapes and macroscale patterns led to the development multichannel designs. This involves creating a defined number of channels within the luminal space of tubular constructs to control axonal target reinnervation⁶⁹. The number of channels has been shown to influence successful repair of rat sciatic nerve defects, with 1 and 4-channel molded collagen-based tubular constructs both supporting excellent axonal regeneration, while only 4-channel tubular constructs were capable of promoting enhanced target reinnervation⁷⁰. Although modifications in the form of intraluminal guidance channels that resemble natural perineurium could reduce axonal dispersion when compared to hollow single-lumen conduits, macroscale luminal architecture cannot provide cell-specific topographical cues for enhanced axonal pathfinding or Schwann cell migration.

2.3.2 Microscale luminal architecture

Subsequent modifications have been made at the microscale level as more advanced fabrication techniques became available. Microscale modifications have the capacity to enhance repair at the cellular level, either by increasing Schwann cell migration efficiency or axonal growth. Guiding microfilaments are long strands of biomaterial bunched up together that may be used on their own or in combination with a tubular construct by incorporating them as a luminal filler. The number of microfilaments has varied with small numbers (8) of

polyamide microfilaments inside silicone-based tubular constructs showing success over small gap repair^{71,72} to larger numbers (2000) of polyglycolic acid microfilaments capable of regenerating gaps of up to 30 mm in dogs⁷³. The latter study also used an outer porous wall that was semi-permeable. The success reported in this study was owed to the combinatorial approach used in terms of the NGC design: its permeability and microscale architectural guidance properties. Collagen-based microfilaments have also been used in biomimetic design showing success bridging 20 mm sciatic nerve gaps in rats, and later 30 mm, in comparison to hollow collagen-based tubular constructs that failed to bridge the defect^{74,75}. The microscale alignment provided by the unidirectionally aligned microfilaments directed axonal pathfinding and enhanced Schwann cell migration. In addition to this, the biomimetic construct also had significantly increased radial permeability, ratifying the importance of this property for success in long gap repair. Advanced lyophilization techniques have led to the development of unidirectionally aligned porous microarchitectures within collagen-based tubular constructs⁷⁶. Longitudinally aligned porous structures are fabricated by controlling the direction of heat transfer in the fabrication process. The use of these structures as luminal fillers in tubular constructs has been demonstrated to enhance their radial permeability and also support unidirectional neural cell migration *in vitro*⁷⁷. Filled tubular constructs with longitudinally aligned microporous collagen-based luminal fillers have had success *in vivo* with the repair of 15 mm gaps in the sciatic nerve of rats, a significant improvement compared to hollow tubular constructs⁶⁵. While the focus of these modifications in the literature has been primarily on enhancing axonal growth and Schwann cell migration, substrates with microscale architecture can also modulate macrophage polarization⁷⁸. It has been shown how microscale patterning can reduce the inflammatory response and limit foreign body reaction⁷⁹. A biochemical strategy has previously been developed to modulate macrophage phenotype by the incorporation of anti-inflammatory cytokines into a tubular construct, leading to enhanced Schwann cell migration and axonal outgrowth⁸⁰. However, it would be interesting to elucidate in future studies whether similar results can be obtained by modulating host macrophages through the incorporation of intraluminal microscale architecture.

2.3.3 Nanoscale luminal architecture

Advances in nanotechnology have led to the possibility of designing architectural patterns at the nanoscale level (1-100 nm). While nanostructures have yet to be incorporated into tubular constructs, studies carried out on 2D substrates with nanoscale properties suggest that they may have a significant influence on nerve regeneration. It has been shown that surface patterning of a size equal to or smaller than the neural progenitor cell soma ($\leq 20 \mu\text{m}$) results in excellent cell alignment and also limits the number of neurites per cell, and thus maximizes neurite length whilst restricting the risk of off-target reinnervation⁸¹. A similar

study examined the effect of nanoscale ridge widths (500-2000 nm) on neural alignment, and found that at this length scale, despite restrictions to focal adhesion formation, neural cells were relatively insensitive to ridge widths with all patterned substrates capable of directing cell alignment in comparison to non-aligned substrates⁸². Interestingly, the density of nanoscale topographical features, which can be controlled, such as post density has been demonstrated to influence the speed and migration distance of melanoma cells, and thus opens the possibility of applying this technique for tubular constructs with nanoscale structural modifications⁸³.

Advances in material fabrication techniques have allowed researchers to develop microscale luminal architectures with enhanced repair capabilities, and recent studies suggest that nanoscale cues may also be incorporated in the next generation of tubular constructs. Combining the pertinent data from these studies allows us to postulate that a combination of micro- and nanoscale alignment channels with suitable post density for focal adhesion formation may allow for directed and also maximal neurite extension solely mediated by topographical cues. A caveat to this approach is that neural differentiation has been shown to be hampered by narrow micropatterning width, and therefore a trade-off with axonal guidance and suitable differentiation topographies must be sought.

2.4 Surface modifications with basal lamina proteins for enhanced repair

Laminin and fibronectin are two basal lamina proteins that are critical in both the development and repair of peripheral nerves due to their association with Schwann cells. Laminin has been shown to modulate the process of myelination, and participates in non-myelinating Schwann cell development⁸⁴. Fibronectin is crucial in the migratory pathways of PNS cells⁸⁵. In 2011 researchers evaluated the effect of modifying collagen-based NGCs with basal lamina proteins. Specifically, collagen microfilaments were crosslinked with laminin and used as a luminal filler in a tubular construct⁸⁶. Modification with laminin led to significantly improved repair across a 5 mm gap in the sciatic nerve of rats. Another study modified a tubular construct with a mixture of fibronectin and laminin, demonstrating recovery of nerve functionality that was comparable to that of autografts across a 10 mm gap in the sciatic nerve of rats⁸⁷. Bioactive amino acid motifs found in laminin and fibronectin have also been used. The amino acid sequence Arg-Gly-Asp (RGD) found in fibronectin, which is similar in function to the Ile-Lys-Val-Ala-Val (IKVAV) sequence in laminin, has been shown to enhance the attachment of migrating Schwann cells across collagen-chitosan-based substrates, leading to the increased regeneration across a 15 mm gap in the sciatic nerve of rats⁸⁸. Taking the pertinent data from these studies, further structural modification of

tubular constructs with basal lamina proteins or bioactive amino acid motifs can significantly improve their repair capacity.

3. Cells for peripheral nerve repair: towards Schwann cell alternatives

Success in peripheral nerve repair is largely dependent on the performance of endogenous Schwann cells specifically at the distal nerve stump. Their natural ability to switch between a homeostatic, myelinating phenotype, and a pro-regenerative, non-myelinating phenotype is essential to their performance¹¹. De-differentiated Schwann cells must be capable of migrating across the site of injury to encourage the formation of bands of Büngner. However, Schwann cells may undergo natural senescence as they migrate across the site of injury, and do so progressively more when met with larger gaps. Autografts perform quite well in part due to the fact they replenish the site of injury with autologous Schwann cells. In this context, researchers have developed strategies to enhance the repair of cell-free or standalone tubular constructs by loading them with Schwann cells – either autologous Schwann cells (ASCs) or stem cells with the plasticity to differentiate into a Schwann cell phenotype. This section systematically reviews some of the cell types used for this purpose (Tab. 1).

Cell type	Pros and cons	NGC type	<i>In vivo</i> assessment	Outcome	Ref
Autologous Schwann cells	Phenotype +++	PGA conduit	Rat facial nerve (5 mm)	Electrophysiological results and nerve regeneration were superior in cell-loaded group	89
	Immunity +++	Collagen conduit	Rat sciatic nerve (20 mm gap)	In all parameters, addition of cells rendered best regeneration	51
	Cost +	Collagen conduit	Rat sciatic nerve (18 mm gap)	Supplementing NGC with cells enhanced regeneration over a distance normally prohibitive	36
	Availability & ease of culture +				
Neural stem cells	Phenotype ++	PLA conduit	Rat sciatic nerve (15 mm gap)	Functional recovery was significantly improved in group with addition of cells	90
	Immunity +	Chitosan conduit	Rabbit facial nerve (10 mm gap)	In all parameters, addition of cells rendered best regeneration	91
	Cost +	Collagen conduit	Rabbit facial nerve (5 mm gap)	Significant improvement observed when injury bridged with NGC with implanted cells	92
	Availability & ease of culture +	Silicone conduit	Rat sciatic nerve (15 mm gap)	Implanted cells differentiated into supportive cells and contributed to axonal regeneration	93
Bone marrow stem cells	Phenotype ++ Immunity +++ Cost ++ Availability & ease of culture ++	Allograft	Rat sciatic nerve (15 mm gap)	Cells helped improve nerve regeneration and functional recovery	94
		PLCL conduit	Rat sciatic nerve (12 mm gap)	Cells promoted axonal regeneration, secretion of neurotrophic factors, and nerve angiogenesis	95
		Chitosan conduit	Monkey median nerve (50 mm gap)	Nerve regeneration and functional recovery was far more efficient in cell-loaded NGCs	96
		Silicone conduit	Rat facial nerve (3 mm gap)	Cells helped improve nerve regeneration and functional recovery	97
		Xenograft	Rat sciatic nerve (10 mm gap)	Nerve regeneration and functional rehabilitation was advanced significantly over acellular group	98
		Matrigel graft	Rat sciatic nerve (15 mm gap)	Cells were able to differentiate into myelinating cells, capable of supporting nerve fibre re-growth	99
Adipose stem cells	Phenotype ++	Fibrin glue conduit	Rat sciatic nerve (10 mm gap)	Cells improved axonal and fibre diameter and functional regeneration	100
	Immunity +++	Vein graft	Rat sciatic nerve (10 mm gap)	Adipose derived stem cells showed enhanced regeneration similar to BMSCs	101

	Cost ++ Availability & ease of culture +++	Allogenic artery	Rat facial nerve (8 mm gap)	Better functional improvement, axonal growth, and improved target reinnervation in cell-loaded group	102
		Xenograft	Rat sciatic nerve (10 mm gap)	Cells aligned along longitudinal axis of graft, and expressed a plethora of neurotrophic factors	103
		PHB conduit	Rat sciatic nerve (10 mm gap)	Results suggest beneficial effect is due to release of growth factors from transplanted cells	104
Skin stem cells	Phenotype ++ Immunity +++ Cost ++ Availability & ease of culture ++	Allograft	Rat sciatic and nerve (12 mm gap)	Cell therapy improved functional repair	105
		Nerve graft	Rat sciatic nerve (12 mm gap)	Significant improvement in histomorphometry and functional results due to cell loading	106
		Collagen conduit	Rat sciatic nerve (16 mm gap)	Recovery rate and functional index was found to be higher in animals that received cell therapy	107
Embryonic stem cells	Phenotype ++ Immunity + Cost + Availability & ease of culture +	Direct injection	Mouse sciatic nerve (2 mm gap)	Transplantation of cells in sphere supported enhanced regeneration over single cell suspension	108
		Epineurium conduit	Rat sciatic nerve (10 mm gap)	Cells differentiated into myelin producing cells, which supported nerve regeneration	109
Induced pluripotent stem cells	Phenotype ++ Immunity +++ Cost ++ Availability & ease of culture ++	PLA/PCL conduit	Mouse sciatic nerve (5 mm gap)	Nerve regeneration was accelerated by the addition of cells	110
		PLA/PCL conduit	Mouse sciatic nerve (5 mm gap)	In all parameters, addition of cells rendered best regeneration	111
Genetically modified (GM) cells	Phenotype + Immunity ++ Cost + Availability & ease of culture +	Allograft + GM autologous Schwann cells	Rat sciatic nerve (14 mm gap)	Permanent overexpression of neurotrophic factors prevented functional regeneration and caused axon entrapment	112
		n/a	<i>In vitro</i> transwell system	c-Jun modified Schwann cells enhanced native Schwann cell migration and promoted their proliferation in the presence of axons	113

Table 1. Successes in cell-loaded NGC-based therapies for peripheral nerve repair

The pros and cons associated with each cell type are depicted in terms of their phenotype compared to autologous Schwann cells (+ = different, +++ = very similar), their likelihood to elicit an immune response when transplanted (+ = quite likely, +++ = unlikely), the relative cost associated with obtaining them (+ = high, +++ = low), and in terms of their relative availability and ease of manipulation, culture and passaging (+ = difficult, +++ = easy). Studies are sorted by cell type criterion including both autologous Schwann cells and stem cells currently in use by researchers. The table also includes NGC or graft type, the relevant *in vivo* experimental assessment conducted in each case, and the outcome of the studies.

3.1 Autologous Schwann cells for enhanced, but costly repair

The goal of using autologous Schwann cells (ASCs) is to enhance the repair capacity of tubular constructs by replenishing senescent Schwann cells at the site of injury. This concept formed the basis of studies comparing tubular constructs without cells (cell-free) with those incorporating them (cell-loaded). The incorporation of ASCs has been demonstrated to enhance repair across an 18 mm gap in the sciatic nerve of rats compared to cell-free constructs³⁶. An advantage of autologous Schwann cells is that they are unlikely to elicit an immune response after transplantation, an issue related with the use of other cell sources. The number of transplanted cells has been identified as an important factor – whereby too

few cells may result in delayed and limited regeneration, while a cell number greater than 500,000 has been shown to have a significantly more potent effect^{36,51}. While it is difficult to monitor transplanted cells *in vivo*, it has been suggested that ASCs act by enhancing the conduction of electrical impulses through myelination of axons, by providing neurotrophic support for both motor and sensory axons through the release of bioactive signals, and by contributing to the production and maintenance of the basal lamina¹¹⁴. Limitations associated with the use of ASCs include an invasive procedure during harvest with a limit on the number of cells that can be extracted, and isolation and subsequent culture of extracted cells for expansion is both a costly and time consuming procedure in a situation where early intervention is critical. In contrast to this, advances in stem cell technologies have presented alternatives to ASCs without the associated limitations.

3.2 Stem cells as an alternative to ASCs for enhanced repair

A wide variety of stem cells have been investigated for their use in peripheral nerve repair including neural stem cells⁹², mesenchymal stem cells – bone marrow and adipose tissue derived^{96,101}, skin stem cells¹⁰⁵, embryonic stem cells¹⁰⁹, induced pluripotent stem cells¹¹¹, and genetically modified cells. Although differences exist in stem cell origin, they are all used as alternatives to ASCs.

3.2.1 Neural stem cells

Neural stem cells are an obvious stem cell choice for researchers as they have a propensity to differentiate into Schwann cells⁹³. Cells isolated from rat hippocampus were first found to have the capacity to differentiate into Schwann like cells *in vitro*. Subsequently, their ability to regenerate a 15 mm gap in the sciatic nerve of adult rats was assessed in combination with a collagen-based tubular construct and findings suggest that they were responsible for significantly enhancing the axonal regeneration in comparison to cell-free tubular constructs⁹³. These findings were ratified by another study that found they could also enhance the regenerative capacity of cell-free tubular constructs in a rabbit model⁹². Taken together, these studies highlight the potential therapeutic use of neural stem cells for repair. However, clinical procedures do not currently exist for harvest of neural stem cells from human adult brain tissue, so the use of these cells would be limited to xenogeneic sources which are associated with a high likelihood of eliciting an immune response, thus raising the overall cost of a potential treatment with these cells due to the required use of immunosuppressants.

3.2.2 Bone marrow derived mesenchymal stem cells

Another stem cell alternative includes bone marrow mesenchymal stem cells, which are multipotent and can be differentiated prior to transplantation. One of their biggest advantages in their use is that they can be isolated from human adult tissue, typically from

the marrow of the hip and the sternum – thus they can be harvested from an autologous source, minimizing the risk of eliciting an immune reaction. These cells have the potential to differentiate into tissues other than those of mesoderm origin. For example, they have been successfully differentiated into a Schwann cell phenotype *in vitro* with beta-mercaptoethanol and retinoic acid, and later their ability to enhance repair *in vivo* when used in combination with tubular constructs was demonstrated in the repair of sciatic nerve in rats⁹⁹. Based on this evidence, a study evaluated the long term outcome of a 50 mm long median nerve defect in rhesus monkeys after treatment with natural polymer-based NGCs in combination with bone marrow derived mesenchymal stem cells, finding that cell-loaded tubular constructs performed significantly better compared to cell-free ones⁹⁶. The positive outcomes from this study involving a primate animal model that closely resembles humans are truly a testament to the functional potential that bone marrow mesenchymal stem cells have in long gap repair.

3.2.3 Adipose tissue derived mesenchymal stem cells

Other sources of mesenchymal stem cells such as adipose tissue can also be used for nerve repair. The advantage of this approach is that from 1 g of adipose tissue 5000 cells can be isolated, which is 500 times more cells than from an equivalent amount of bone marrow¹¹⁵. Thus, in addition to having the option of obtaining them from an autologous source, they also come with a relatively higher availability than other cell types. These cells have been used in combination with tubular constructs either as undifferentiated cells¹⁰⁴ or by differentiating the cells *in vitro* prior to implantation *in vivo*¹⁰³, with both approaches showing promising results. In either case, it has been suggested that these cells evoke an early response, lasting less than 14 days, suggesting that the regenerative effect observed in the repair of a 10 mm sciatic nerve defects is mediated by an initial boost of released neurotrophic factors in the regenerative microenvironment^{103,104}.

Lesser used adult stem cell sources for nerve repair include the skin and hair follicles. For instance, precursor skin derived stem cells used in combination with tubular constructs have been shown to enhance the repair across both sciatic and tibial nerves in the rat¹⁰⁵⁻¹⁰⁷.

3.2.4 Embryonic stem cells

Despite the ethical considerations surrounding the harvest and use of embryonic stem cells, their application in nerve repair is significant. Embryonic stem cells hold great potential since they can differentiate into all three germ layers including the ectoderm, which matures into neural tissue during development. In 2008 researchers evaluated their performance by firstly differentiating them *in vitro* towards a neural lineage and then enhanced the repair of rat sciatic nerve when used in combination with a tubular construct¹⁰⁹. Another study investigated the manner by which they were loaded – either in single cell suspension or as neurospheres – with findings suggesting that loading embryonic stem cells into tubular

constructs as neurospheres results in significantly improved repair than when loaded as single cell suspension, possibly due to increased levels of neurotrophic factor production observed in the supernatant of cells bound to each other in sphere cultures¹⁰⁸. However, in addition to having ethical complications, the cells would also have to be harvested from allogenic tissue, and thus they would be more likely to elicit an immune response than autologous cells.

3.2.5 Induced pluripotent stem cells

A similar approach involves the use of induced pluripotent stem cells, which are adult cells that have been genetically re-programmed to have pluripotency. The advantage of this cell type is that they can be isolated from human adult tissues and have similar properties to embryonic stem cells. However, they are relatively more costly to culture since they require to be re-programmed *in vitro* prior with Yamanaka factors prior to transplantation. They have previously been used in combination with tubular constructs and incorporated as neurospheres^{110,111}. Functional outcomes are similar to those observed with embryonic stem cells, for example the combination of induced pluripotent stem cells with tubular constructs led to enhanced repair in the sciatic nerve of rats¹¹⁰. Although there are risks associated with the use of these cells such as tumor formation¹¹⁶ that must be overcome, they do not have the same ethical implications associated with the use of embryonic stem cells, making induced pluripotent stem cells the more viable choice from a regulatory perspective.

3.2.6 Genetically modified cells

A lesser used approach involves genetically modified Schwann cells, which typically overexpress a pro-regenerative cytokine, or neurotrophic factor of interest – thus their phenotypes differ quite considerably to that of autologous Schwann cells. For examples, in a recent study Schwann cells were isolated *in vitro* and infected with a lentiviral vector to induce glial derived neurotrophic factor overexpression¹¹². Although results from the repair of 14 mm gap in the sciatic nerve of rats treated with an allograft supplemented with these cells were promising at an early time point, permanent overexpression was ultimately detrimental to regeneration and resulted in aberrant repair. Multiple cytokines can also be overexpressed simultaneously by transfecting Schwann cells with a single gene encoding for a transcription factor involved in the regulation of a plethora of neurotrophic factors – cJun²¹ – leading to significant overexpression of multiple neurotrophic factors and enhanced Schwann cell migration and proliferation *in vitro*¹¹³. Overall, the concept of genetically modified is promising, but further studies investigating the use of more transient gene expression profiles that modulate the therapeutic release of overexpressed cytokines will be needed in order to overcome some current limitations with this approach.

4. Neurotrophic factors for peripheral nerve repair: towards targeted therapeutics

Significant ongoing research is focused on enhancing the repair capacity of NGCs by incorporating biochemical neurotrophic factors instead of cells. Neurotrophic factors are biomolecules that signal neural cells and are naturally involved in supporting their growth, survival and differentiation. Historically, the first neurotrophic factor to be described was nerve growth factor (NGF) in 1951¹¹⁷. Two mouse sarcomas were found to have stimulating effects on nerve growth and based on these observations it was suggested that a 'diffusible nerve promoting agent' (later to be known as NGF) was responsible for these growth-stimulating effects. This discovery was followed by that of other neurotrophic factors including brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin 4/5 (NT-4/5)¹¹⁸⁻¹²⁰. Neurotrophic factors have since been described by their specific biological activity, which is defined by the receptor that allows them to bind to cells. Their tertiary amino acid configuration has the capacity to bind to tropomyosin-related kinase (trk) receptors found on the surface of target neural cells – some with a higher affinity than others leading to their distinct and cell-specific bioactivities, as summarized in Table 2.

Growth factors	Target cell	Specific receptor	Bioactive effect	Reference
NGF	Sensory & sympathetic neurons	trkA	Promotes survival and growth	121
BDNF	Sensory and motor neurons	trkB	Promotes growth and synapses of neurons	122
NT-3	Sensory neurons	trkC	Neurogenesis and gliogenesis	123
NT-4/5	Sensory neurons	trkB	Similar to BDNF	124
GDNF	Schwann cells and motor and sensory neurons	GFR α	Stimulates proliferation and promotes survival	125-127
CNTF	Sensory and motor neurons	CNTF-R	Promotes survival	128
TGF β 1	Sensory neurons	TGF β -R	Promotes survival	129
bFGF	Sensory neurons	FGF-R	Supports neurite outgrowth	130,131

Table 2. Biological activity of neurotrophic factors

This table summarizes the interaction between neurotrophic factors and target receptors expressed on the surface of neural cells. They include neurotrophins: nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5); glial derived neurotrophic factor family ligands: namely glial derived neurotrophic factor (GDNF); and neurotrophic cytokines such as ciliary neurotrophic factor (CNTF). Other factors that have been shown to exhibit pseudo-neurotrophic behavior include transforming growth factor (TGF β 1) and basic fibroblast growth factor (bFGF). Also listed are the cell types that can respond to specific neurotrophic factors, the corresponding receptors, and the associated biological effect.

In healthy nerves, neurotrophic factors play a homeostatic role in the natural turnover of neural cells and are produced predominantly by Schwann cells. However, in response to injury there is a significant upregulation in the production of many of the listed biomolecules. Based on this rationale the strategy of targeted therapeutics for peripheral nerve repair has emerged. It typically involves delivering a dose of exogenous neurotrophic factor(s) within a tubular construct in order to enhance nerve repair. While in this section we provide an

overview of the considerations required for the use of some specific neurotrophic factors, we direct the reader towards the following review for a more in-depth comparison of the relevant neurotrophic factors¹³².

4.1 Controlled neurotrophic factor release is pivotal for effective repair

Studies have focused on controlling the temporal profile of exogenous neurotrophic factors. Firstly, this avoids presenting cells with excessive amounts of the neurotrophic factor, or overdose, which may lead to aberrant repair. Secondly, controlling the temporal profile enhances long term repair by providing cells with sustained doses of neurotrophic factors over a longer period of time, in ways mimicking the natural function of Schwann cells. Thirdly, this ensures that the right cells receive their dose of neurotrophic factor when they need it most. The tubular constructs used for neurotrophic factor delivery can determine the means by which they are released. For example, both GDNF and NGF were loaded into a silk fibroin-based NGC during its fabrication step, resulting in a temporal profile of continuous release over 28 days, which was largely controlled by the ionic interaction between the positively charged neurotrophic factors and the negatively charged silk fibroin⁵⁹. Alternatively, controlling the temporal profile can be dependent on an intermediate between the neurotrophic factor and the NGC such as a microparticle-based delivery system. For example, poly-lactic-go-glycolic-acid (PLGA) microparticles loaded with NGF have been incorporated into tubular constructs to successfully repair sciatic nerve defects in rats^{133,134}. Similarly, another study investigated the regenerative effect of PLGA microparticles loaded with NGF, BDNF, GDNF or FGF-2, incorporated into tubular constructs made up of silicon and collagen, and found that slow and controlled release of this neurotrophic factors led to enhanced repair¹³⁵. Another study compared two PLGA microparticle formulations, achieving controlled release of GDNF either in 2-weeks or 4-weeks by adjusting the molecular weight of PLGA used in the formulation, finding a significant improvement over negative controls and no significant difference compared to isograft treatment of a 10 mm gap in rats when used in combination with an allograft¹³⁶. These microparticle-based delivery systems depend on the tunable degradation properties of microparticles to release the neurotrophic factor dose when needed, and offer a viable approach from a controlled release perspective.

4.2 Regulating neurotrophic factor dose is key for effective and non-aberrant repair

Once the neurotrophic factor(s) have been selected, the dose or therapeutic quantity delivered in the construct should be carefully considered. While a low dose can have little therapeutic benefit, a high dose can lead to aberrant axonal regeneration, poor functional results, and off-target effects in addition to being costly¹³⁷. For example, high levels of GDNF within a site of peripheral nerve injury can lead to axon entrapment and decrease the

number of axons extending into the distal nerve stump and beyond to correct reinnervation¹¹². There is disagreement regarding the exact mechanism that controls axon entrapment and two mechanisms of action have been suggested in the literature. The first is due to the biochemical 'pull' that high concentrations of neurotrophic factors such as GDNF exert on axons^{138,139}. It has been suggested that while this biochemical 'pull' remains present in the site of injury axons will be entrapped. The second is due to Schwann cell overexposure to neurotrophic factors which may induce a non-regulated hyper-response from them, preventing axons from exiting the site of injury¹²⁵. Few studies have systematically compared low and high doses of neurotrophic factors, but they highlight the importance of avoiding non-aberrant repair due to overdose. For example, it has been demonstrated that low doses (0.5-2 µg/day for 28 days) of directly applied BDNF can facilitate axonal regeneration of motor neurons while to high doses (12-20 µg/day for 28 days) can significantly inhibit axonal regeneration¹⁴⁰. However, recent studies have emphasised how even lower doses of neurotrophic factors are more advantageous for peripheral nerve repair by enhancing regeneration and limiting aberrant effects in tandem. Controlled release of GDNF and NGF in the range of 1-10 ng from silk fibroin-based constructs has been suggested to be optimal for nerve repair¹⁴¹. Similarly, another study used a 20 ng/day dose of BDNF in the treatment of a sciatic nerve injury in rats, showing significant improvement in axonal growth and protection from atrophy after injury¹⁴². Another factor to consider regarding dosage is the concentration gradient across the site of injury. It has long been established how axons turn towards high concentrations of neurotrophic factor in a process referred to as chemotaxis¹⁴³. Bellamkonda has investigated the effect of anisotropic distribution of neurotrophic factors (a gradient of NGF concentration in tubular constructs: low at proximal end, high at distal end), with results showing how anisotropic distribution leads to enhanced regeneration compared to isotropic distributions across a 20 mm sciatic nerve gap^{144,145}. Thus, it is clear that future strategies for NGC development must consider not only the specific NFs to incorporate but also the spatial and temporal control of their presentation to enhance regeneration

4.3 Co-delivery of neurotrophic factors can lead to synergistic effects

Successful repair requires a complex interplay between Schwann cells, sensory and motor neurons. Given this and the cell-specific bioactivity of neurotrophic factors, some studies have focused on co-delivery of neurotrophic factors in combination with tubular constructs in order to synergistically enhance the overall therapeutic effect¹⁴¹. For example, it has been demonstrated both *in vitro* and *in vivo* that NGF promotes the survival and axonal outgrowth of sensory neurons¹⁴⁶, while GDNF is an important survival factor of motor neurons and sensory neurons¹⁴⁷, and stimulates the migration and proliferation of Schwann cells¹²⁵.

Based on these findings, three neural cell types (sensory neurons, motor neurons and Schwann cells) might potentially be targeted with two neurotrophic factors (NGF and GDNF)⁵⁹. The co-delivery of NGF and GDNF via silk-fibroin tubular constructs has been shown to synergistically promote enhanced neural cell activity⁵⁹. Similar studies¹⁴⁸⁻¹⁵⁰ have also highlighted additive and synergistic effects observed with other neurotrophic factor combinations. For instance, the combination of NGF and IGF-1 (Insulin-like growth factor 1) has been shown to synergistically enhance the growth and elongation of axons in dorsal root ganglia, with evidence suggesting that convergence within the PI 3-K-Akt-GSK-3 pathway is responsible for the observed synergism¹⁵⁰.

5. Concluding remarks: From next generation therapeutics to clinical translatability

Currently approved NGCs are generally hollow tubular constructs with biodegradable properties such as Neuragen¹⁵¹, Neurotube¹⁵², Neurolac¹⁵³ and Neuroflex³⁰, which have been approved by the Food and Drug Administration (FDA) in the US for PNR (Tab. 3). Only a small fraction of patients with PNI have benefited from treatment with these NGCs because of their limited capacity to repair larger gaps efficiently. To overcome this limitation, NeuraGen 3D has been developed with a collagen-based luminal filler, showing an improvement compared to hollow constructs and no significant difference to autografts in the repair of small, 10 mm, gaps⁶⁵. However, when it was evaluated in a larger rabbit peroneal 30 mm gap model, the autograft performed significantly better than the filled construct²⁴. This highlights how filled conduits present an improvement over hollow conduits but only up to a certain gap length, beyond which the autograft performs significantly better. Alternatively, AxoGen Inc.'s Avance Nerve Graft, an acellular human nerve allograft sourced from donated peripheral nerve tissue and processed in accordance to the FDA requirements for human cellular and tissue-based products, has demonstrated superior clinical outcomes compared to approved NGCs in animal models¹⁵⁴. In a clinical study, 10 PNIs were treated with an average graft length of 22.3 mm, with results showing adequate return of sensation in as little as 9 months post-op and no aberrant side effects such as infection or rejection were observed¹⁵⁵. A recent study by Poppler *et al.*, assessed their capacity to regenerate longer defects > 3 cm, where autografts would typically be prescribed, and suggests a reason for axonal growth arrest in longer gaps. Results of this study suggest that longer allografts are repopulated with Schwann cells that have accumulated much greater levels of senescence markers when compared to shorter < 3 cm allografts, in addition to higher stromal cell presence¹⁵⁶. Therefore, the primary focus for the healing of long gaps should focus on preventing cellular senescence. Some potential molecular targets to achieve this include *Jag1*, which is involved in activating the Notch signalling pathway and regulates gene expression to control Schwann cell dedifferentiation and proliferation¹⁵⁷. Alternatively, c-Jun,

which has been shown to be a major regulator of Schwann cell phenotype, is regulated by the ERK signalling pathway also has potential to be targeted¹⁵⁸. We direct the reader towards the following review, which discusses other interesting potential more in-depth¹⁵⁹. Collectively these studies highlight that commercially available NGCs are currently only best for small gap repair, allografts present an alternative up to a certain point beyond which autografts remain the clinical gold standard. However, stem cell-based products and neurotrophic factor containing tubular constructs have yet to be approved by regulatory agencies for use in nerve repair and could provide an improvement over currently available treatments.

FDA approved NGC	Composition	Clinical study & outcomes		Ref
NeuraGen 3D Integra LifeSciences Approved in 2014	Filled tubular construct with outer wall made of collagen type I and luminal filler made of collagen-glycosaminoglycan	Sciatic nerve (animal study) 10 mm gap	Significant improvement over hollow constructs, axonal regeneration same as autograft	65
NeuraGen Integra LifeSciences Approved in 2001	Hollow tubular construct collagen type I	Alveolar nerves, used as nerve cuff – no gap	Only half of patients with 'good improvement'	28,151,160
		Digital nerve, 12.5 mm gap	4/12 patients excellent sensibility, 5/12 good, 1/12 poor, 2/12 no recovery	
		Median nerve, 20 mm gap	Neuroma formation reported	
Neurotube Synovis Approved in 1999	Hollow tubular construct polyglycolic acid	Median nerve, 30 mm	Good recovery of motor and sensory function	152,161
		Digital nerve, <4 mm gap	Good recovery, however no statistical significance between conduit and control group	
Neurolac Polyganics Inc. Approved in 2003	Hollow tubular construct poly dl-lactide-co-ε-caprolactone	Digital nerve, <5 mm gap	Good recovery, however no statistical significance between conduit and control group	30,153
		Sciatic nerve (animal study), 10 mm gap	Issues with swelling, degradation rate and auto-mutilation	
NeuroMatrix & Neuroflex Collagen Matrix, Inc. Approved in 2001	Hollow tubular construct collagen type I	No publications available on performance, recommended for gaps less than 25 mm in length		30

Table 3. FDA approved nerve guidance conduits for peripheral nerve repair

The table provides a list of FDA approved NGCs for use in peripheral nerve repair, and does not include processed allografts such as Avance (Axogen), or nerve wraps (e.g. Salutunnel). Their composition and clinical performance is also summarized in terms of gap length and functional outcomes.

Remarkable advances in the research field have led to the identification of both structural and biochemical components to enhance the repair capacity of hollow NGCs for long gap repair. Significant progress has been made in the early phase of clinical translation. Both functional and morphometric techniques used to assess the performance of NGCs *in vivo* are well established. However, standardization across animal studies in terms of small and

long gap repair models would make clinical translation more efficient and allow more accurate comparisons to be made between studies¹⁶². Studies are increasingly becoming concerned with describing the performance of tubular constructs in comparison to autografts, making them clinically relevant. Many studies have reported that NGCs are performing on par with autografts *in vivo*, and this is particularly evident where biochemical cues, either cells or neurotrophic factors, are incorporated into pre-clinical studies. Moreover, cell-free or neurotrophic factor-free NGCs are generally outperformed by their cell-loaded or neurotrophic factor-loaded counterparts. Despite advances in stem cell technology, the best combination of construct and cell type is yet to be realized. However, the progress in the field suggests there are improvements using cell-based strategies that are to a large extent governed by Schwann cell-mediated cellular activity and neurotrophic factor production. Considering the latter, an interesting question posed by this review is whether the same beneficial effect of cell-loaded constructs can be achieved by delivering neurotrophic factors in cell-free NGCs. Researchers have identified two important parameters pertaining to neurotrophic factor delivery – controlled release and dose. While neurotrophic factor release kinetics from controlled delivery systems and dose can be highly optimized *in vitro*, the translation of such systems *in vivo* is difficult to monitor and is often assessed by the gross performance of neurotrophic factor loaded constructs in terms of their regenerative capacity compared to neurotrophic factor-free tubular constructs. Another caveat of a neurotrophic factor delivery approach is their cell-specific nature since successful repair requires more than just a single cell type to have their cellular activity enhanced. Tandem delivery of neurotrophic factors overcomes this but incurs significant costs. Conversely, their specificity for receptors expressed on the surface of neuronal cells prevents them from non-specific side effects and enhances therapeutic control. Therefore, while the progress with neurotrophic factor-based approaches illustrated in this review is promising, further research is required to fully exploit their potential while optimising their cost-benefit balance.

To conclude, while there is significant research ongoing in the field and new technologies are continuously emerging, further research and an increased focus on overcoming the regulatory barriers associated with translating such technologies to the clinic is required before next generation nerve guidance conduit-based therapeutics will replace the gold standard of autologous nerve grafts.

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Advances in nerve guidance conduit-based therapeutics for peripheral nerve repair

Authors

[William Lackington](#)^{1,2,3}, [Alan Ryan](#)^{1,2,3}, and [Fergal O'Brien](#)^{1,2,3*}

¹Tissue Engineering Research Group (TERG), Department of Anatomy, Royal College of Surgeons in Ireland.

²Advanced Materials and BioEngineering Research (AMBER), Trinity College Dublin, Ireland.

³Trinity Centre for BioEngineering (TCBE), Trinity College Dublin, Ireland.

*Phone +353 1 402 2149, E-mail: fobrien@rcsi.ie

Graphic

