Neural Guidance Conduits in Peripheral Nerve Repair: A Contemporary Alternative to Autografts

Research minor Literature review

Kaia L. M. Pauli
Student number 645608
Bachelor of Applied Sciences in Life Sciences
KLM.Pauli@student.han.nl

Host Organization:
Life Sciences Research Institute
Dalhousie University
1344 Summer Street
Halifax, NS B3H 0A8
Canada

Host Supervisor:
Prof. Dr. John P. Frampton
Associate Professor
School of Biomedical Engineering
Dalhousie University
Email: John.Frampton@dal.ca

Home Organization:
HAN University of Applied Sciences
Laan van Scheut 2,
6525 EM Nijmegen,
Netherlands

Home Supervisor:
Kelly Vellinga Chan
Lecturer
HAN University of Applied Sciences
Email: K.VellingaChan@han.nl

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2 Abbreviations

CNS	Central Nervous System
ECM	Extracellular Matrix
FN	Fibronectin
NGC	Neural Guidance Conduit
PNI	Peripheral Nerve Injuries
PNR	Peripheral Nerve Regeneration
PNS	Peripheral Nervous System
SC	Schwann Cell
WD	Wallerian Degeneration

3 Abstract

Peripheral nerve injuries (PNIs) are both prevalent throughout society and challenging to treat, especially when the injury is large (> 5mm). When treated unsuccessfully, PNIs often result in significantly diminished quality of life, providing strong motivation for finding reliable treatment procedures. Currently, the preferred method of treatment (autografts) struggles to bridge larger severed nerves gap and is associated with numerous limitations, such as immunological rejection. In this review, we explore an alternative approach that makes use of neural guidance conduits (NGCs), which emulate the natural environment of developing peripheral nerves and serve as a supportive structure for regeneration. As peripheral nerve cells interact strongly with their immediate surroundings (i.e., their extracellular matrix (ECM)) throughout growth, providing such a support can, in principle, enable directionally selective nerve regrowth. Specifically, we review the growth and regeneration of the PNS and contextualize how different ECM molecules influence nerve growth in the PNS. Select NGC designs are discussed, with emphasis placed on how design criteria affect the regenerative process. Though they have not yet become the *de facto* treatment approach for PNI treatment, the elegance and flexibility offered by NGCs suggest that they will play a crucial role in tissue-engineering-based repairs in the near future.

4 Introduction

Peripheral nerve injuries (PNI) are prevalent throughout society, accounting for around 2% of all trauma cases (Wilcox et al., 2020). In contrast to the central nervous system (CNS), the body's "command centre," the peripheral nervous system (PNS), which relays information to the CNS, has some capacity to regenerate following injury (Contreras et al., 2022; Illis, 2012). However, the rate at which the PNS regenerates is disproportional to the size of the majority of PNIs (Contreras et al., 2022). Consequently, most peripheral nerves are not able to reinnervate themselves back to their original state without intervention following axotomy. This results in debilitation, loss of sensation and movement, as well as chronic pain in severe cases. The culmination of these outcomes generally imparts significant socio-economic ramifications in those affected (Wilcox et al., 2020; Wojtkiewicz et al., 2015), leading to a diminished quality of life.

Currently, the "gold standard" approach for treating PNIs are autografts, where a person's intact nerves are used as replacements for the injured tissues. Despite their success in bridging various lengths of lesions, they are wrought with limitations and are not always a viable solution. Consequently, there is currently intense effort invested in finding improved treatment protocols, which range from gene therapy to the use of hydrogels, to facilitate regeneration through their matrix. A particularly promising avenue is the use of neural guidance conduits (NGCs), which are cylindrical "skeletons" with internal scaffolding used to guide and fuse damaged peripheral nerves. For example, NGCs have shown success in bridging smaller nerve transections with success rates comparable to that of autografts (Lackington et al., 2017; Mankavi et al., 2023; Stocco et al., 2023). As such, NGCs are intended to act as "bridges" between the severed nerve endings, ultimately enabling the cells to grow across facilitating target reinnervation and functional recovery.

The concept of simply adding a "bridge" to facilitate peripheral nerve repair (PNR) Is very appealing; however, in practice, it is difficult to achieve due to the inherent complexity of the nervous system and its surroundings (i.e., many facades of interactions, cues and responses that are poorly understood, etc.). In assessing the feasibility of NGCs for PNR, particular emphasis is placed on the interactions between cells and their surrounding environment. It is well-known that peripheral nerve cells expand their network and grow (or regrow) by interacting with their environment. Specifically, the extracellular matrix (ECM) acts as chemical guides for these cells where different constituents elicit responses and growth patterns in the expanding neurons (Gonzalez-Perez et al., 2013). Understanding how these constituents affect the regeneration process is key to finding suitable approaches to assisting in guiding peripheral nerves towards their target.

In this review, we summarize how the PNS expands, regenerates and how regenerative capacities differ in the human nervous system. Thereafter, we summarize the strengths and limitations of the current "gold standard" method (autografts), followed the design and implementation of NGCs. Finally, we briefly discuss how ECM molecules influence cell behaviour

on NGC scaffolds, to better understand the regenerative process that peripheral nerves undergo following axotomy.

5 Overview of the Central and Peripheral Nervous Systems

Although the human nervous system is rather complex, it can anatomically be divided into two components: the CNS and the PNS. Simply put, the CNS consists of the brain and spinal cord, which function together to analyse and integrate both sensory and motor information (Partridge, 1973). On the other hand, the PNS is comprised of sensory and motor neurons that connect the rest of the body to the CNS, enabling sensory perception, movements, and other autonomic functions (e.g., pain, walking, beating heart, etc.) (Purves et al., 2004).

In general, neurons are highly specialized "excitable" cells that integrate and transmit information through electrochemical signalling (Franze & Guck, 2010). They are part of a dynamic (and extensive) network of cells that, for the most part, are actively growing (Yurchenko et al., 2021). As our focus here is on peripheral nerves, their mechanism of growth is of great interest. In this respect, the CNS and PNS exhibit many similarities. For example, they both utilize similar signalling pathways, but differ in terms of how (and when) these pathways are activated or inhibited. Exemplary of this is the JACK/STAT signalling route (involving, among other responses, inflammation), which is activated by the mammalian target rapamycin (mTOR) and is known to promote neural growth (Contreras et al., 2022). In the PNS, mTOR is activated following damage, which is known to enhance the ability of axonal growth (Abe et al., 2010). In contrast, in the CNS, mTOR remains inactive following an injury, contributing to failed regeneration. Interestingly, when the activation of mTOR is forced (e.g., by signal blocking), the growth of corticospinal and retinal ganglion axons is found to be stimulated (Contreras et al., 2022). This highlights both the differences between nervous systems and the complexity of the interactions taking place, as activation or inhibition of even individual proteins within specific pathways can contribute to differential neural growth patterns.

5.1 Neural growth in the PNS

The general structure of a peripheral nerve follows a hierarchical structure consisting of three layers: epineurium, perineurium, and endoneurium (Figure 1). The epineurium comprises the outermost layer and acts as a barrier to the nerve from the surrounding environment. It consists of loose connective tissue that contain blood vessels providing the nerve with nutrients. Within this layer are multiple fascicles which are each surrounded in loose connective tissue layers of perineurium. This layer is both thinner and denser, consisting of sheaths of flat perineurial cells surrounded by collagen fiber bundles. Within each fascicle, enwrapping individual axons is a supporting layer of loose connective tissue termed the endoneurium. The endoneurium is composed of basal lamina, which are arranged continually around the axon and its encompassing constituents (e.g., ECM, fibroblasts, etc.), while also occupying the space between individual neurons (Gonzalez-Perez et al., 2013; Mankavi et al., 2023).

The basic “unit” of a nerve is the neuron, consisting of a cell body, axon, and dendrites. The axonal region contains the myelin sheath which are interrupted by unmyelinated regions termed nodes of Ranvier. The axon is responsible for transmitting messages from the cell body to the dendrites of other neurons and the myelin aids in insulating the axons, improving the efficiency of signal transmission. Further discussion on neuron functionality can be found elsewhere (Franze & Guck, 2010; He & Jin, 2016).

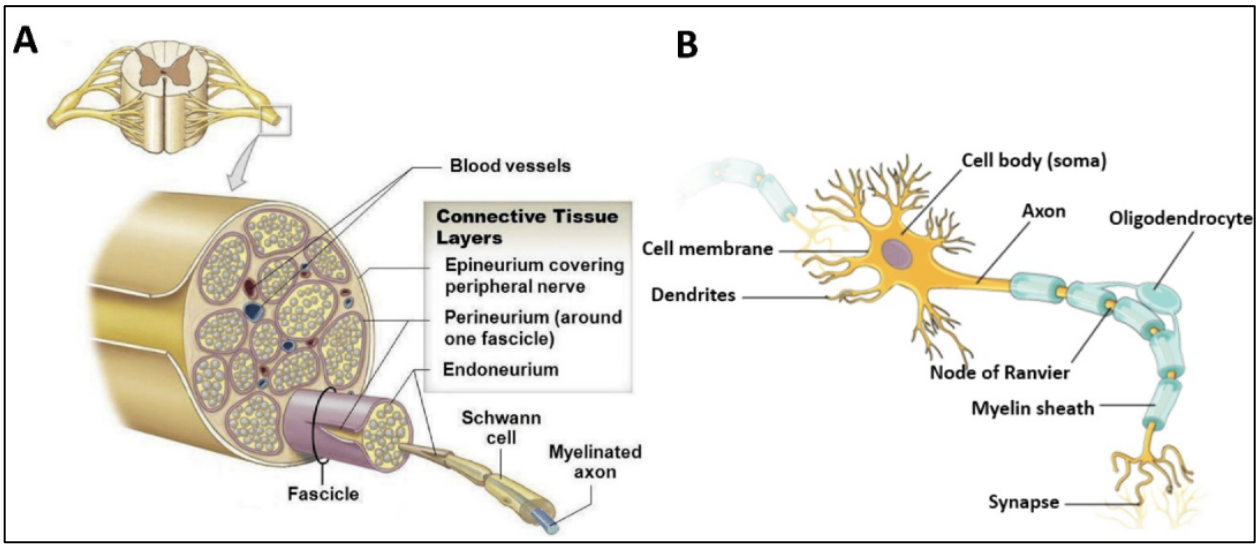

Figure 1: Overview of a nerve’s hierarchy, from its macrostructure down to the cellular level of a neuron. (**A**) A cross-sectional view of a peripheral nerve, highlighting the different connective tissue layers seen throughout their structure. (**B**) The anatomical structure of a neuron. Adapted from (Mankavi et al., 2023).

Studies centred on developing neural cells have elucidated several important regions within the growing tip of an axon (He & Jin, 2016). For example, structures termed growth cones form at the distal tip of a developing axon and aid the structures in expansion and development (Figure 2). At the leading edge of a growth cone, dense fibrillar actin (F-actin) is found, which anchors loose microtubule networks closer to the central axonal domain (He & Jin, 2016; Kolodkin & Tessier-Lavigne, 2013). The polymerization of actin immediately inside the leading-edge aids in creating a forward movement (i.e., directional growth). This actin polymerization allows the membrane to be pushed forwards resulting in membrane protrusions. At the periphery of the growth cone, dense actin-network-forming lamellipodia and filopodia are found (Contreras et al., 2022; Kolodkin & Tessier-Lavigne, 2013). Lamellipodia are fan-shaped membrane protrusions and when these protrusions extend beyond the edge of the extending growth cone, they are termed filopodia (Kolodkin & Tessier-Lavigne, 2013).

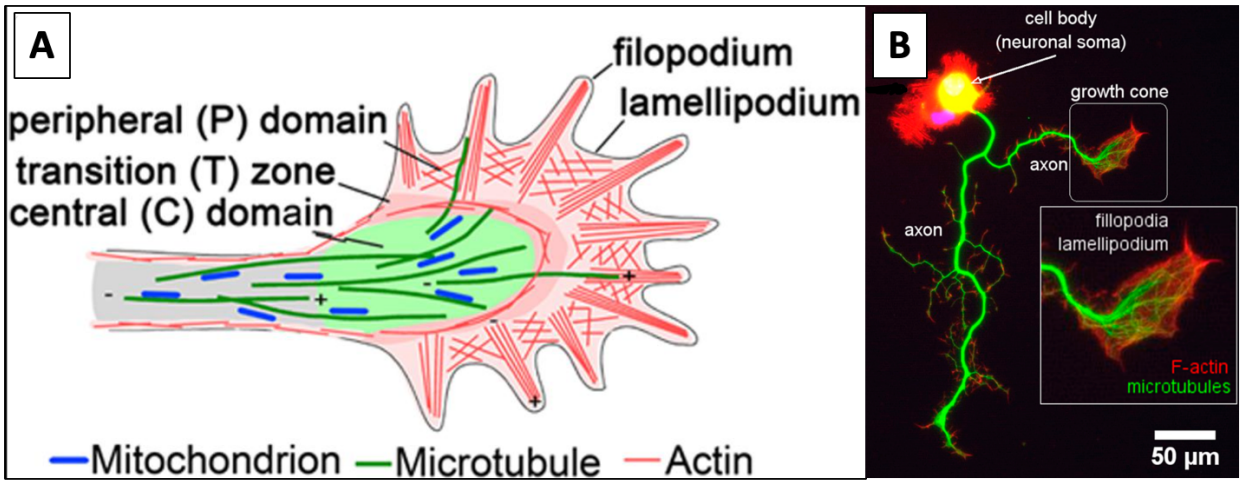

Figure 2: Overview of axonal growth cones. (**A**) Schematic representation of a neuronal growth cone, outlining different regions of cytoplasmic structures, as well as the different regions in which they reside. Adapted from (Miller & Suter, 2018). (**B**) The macrostructure of a growth cone. The neuron is stained with Rhodamine to identify filamentous actin (in red), as well as Phalloidin via anti-tubulin-βIII to identify neural microtubules (in green). On the right side of the figure, the growth cone is highlighted. As part of its structure, filopodia (seen as red protrusions) and lamellipodium (seen as red stained actin in the expanding cytoplasm) are apparent. Adapted from (Muñoz-Lasso et al., 2020).

Healthy growth cones are characterized by the combination of both extensions and retractions of the leading edge of the lamellipodia, as well as the filopodia protrusions (Kolodkin & Tessier-Lavigne, 2013). Following extension, the filopodia require a surface to adhere to, enabling traction-generation within the growth cone, ultimately resulting in tension between the F-actin in the attached projections. Not all filopodia will adhere to their surrounding surface, resulting in retraction of unattached protrusions. Through cycles of extension, attachment, and retractions (i.e., of unattached projections), an overall forward movement is facilitated (Kolodkin & Tessier-Lavigne, 2013). However, the forward motion of an expanding growth cone is also dependent and influenced by the interaction between the permissive surrounding environment and its motile properties, which is discussed in a later section.

Throughout this process, various signalling molecules (e.g., ECM molecules, morphogens, netrins, etc.) act as cues to control the direction of the extending growth cone. Amazingly, the interaction of an individual filopodium with an extracellular target causes the growth cone to adjust the direction of axonal growth (Roy et al., 2013). This not only highlights the importance of the microscale architecture of the surrounding nerve, but also emphasizes the remarkable functionality of the filopodia that guide the growth cones. Associated with larger, more complex growth cones are additional slowly extending cones with numerous branch formations. By contrast, smaller or less mature cones generally extend more rapidly (with less branching) along a more permissive path (Kolodkin & Tessier-Lavigne, 2013).

5.2 Natural nerve repair

Both the CNS and PNS undergo an incredible process following neural damage known as Wallerian degeneration (WD), whereby degeneration and regeneration of the distal axonal region occurs (Figure 3). Despite both nervous systems having this ability, there are key differences that prevent efficient CNS regeneration. Most notably, the rate of WD in the CNS takes months to years, whereas the rate of WD in the PNS ranges from 7-14 days (Vargas & Barres, 2007). This differential rate results in rapid repairs to the PNS where the extracellular environment promotes axon regeneration, whereas the slower WD in the CNS results in the prolonged presence of myelin-associated inhibitors that contribute to failed axonal regeneration (Vargas & Barres, 2007). There are many factors that contribute to these differences in regenerative capacities. Firstly, mature neurons in the PNS are able to regenerate following axotomy, whereas most CNS neurons cannot (Contreras et al., 2022; Illis, 2012). Overall, this is attributed to an imbalance in factors that either inhibit or promote neuronal regeneration in the CNS and PNS, respectively. For example, the CNS lacks upregulation of growth associated genes relative to the PNS, resulting in a poor prognosis (i.e., paralysis) for CNS regeneration (Koenig Editor, 2009). Similarly, the glial cells involved in axonal elongation differ between the two nervous systems. Oligodendrocytes of the CNS exhibit inhibitory effects wherein they express neurite growth inhibitory proteins (e.g., the membrane protein Nogo-A), which inhibit branch formations along maturing axons. In contrast, the Schwann cells (SCs) of the PNS contribute to axonal elongation through stimulatory effects by converting to a repair phenotype that aids in guiding regenerating axons to their target (Gordon, 2016; Jessen & Mirsky, 2016).

In the PNS, the mechanism for WD is both complex and not fully understood. However, it has been well established that peripheral nerves are able to readily regenerate over short distances (< 5mm) (Bai et al., 2015; Rotshenker, 2015). Following transection of a peripheral nerve, a series of molecular and cellular events occur to facilitate distal and proximal segment changes. The proximal portion of the nerve undergoes changes that can vary based on the location and severity of the injury. Proximal segment changes in severe injuries focus more on reprogramming the genetic motive of the cell towards a regenerating phenotype through a process termed chromatolysis. Although the proximal segment doesn't focus as much on the breakdown of its components, there is still the breakdown of the myelin sheath and SC to the adjacent node of Ranvier (Gu et al., 2011).

These events facilitate and trigger a number of distal segment changes including the disintegration of the axoplasmic microtubules and neurofilaments, as well as the later dissolution of the axonal membrane, myelin sheath, and SCs. The dissolution of the axonal components triggers the endoneurium to release chemicals (i.e., histamines and serrations), which attract macrophages, monocytes, and other immune cells to migrate into the degenerating nerve, removing myelin and axon debris (Gu et al., 2011). Simultaneously, SCs proliferate and convert to Büngner SCs, forming longitudinal cell lines (termed Bands of Büngner), which function as regenerating tracks to guide the regenerating axons to their target. Büngner SCs produce neurotrophic factors and ECM molecules, which also aid in guiding axonal sprout growth from the proximal end towards their target. For a nerve to functionally reinnervate itself, it is necessary for

the regenerating axon to elongate through the guidance of growth cones towards their synaptic target (Carroll & Worley, 2017; Gu et al., 2011; Lunn et al., 1989).

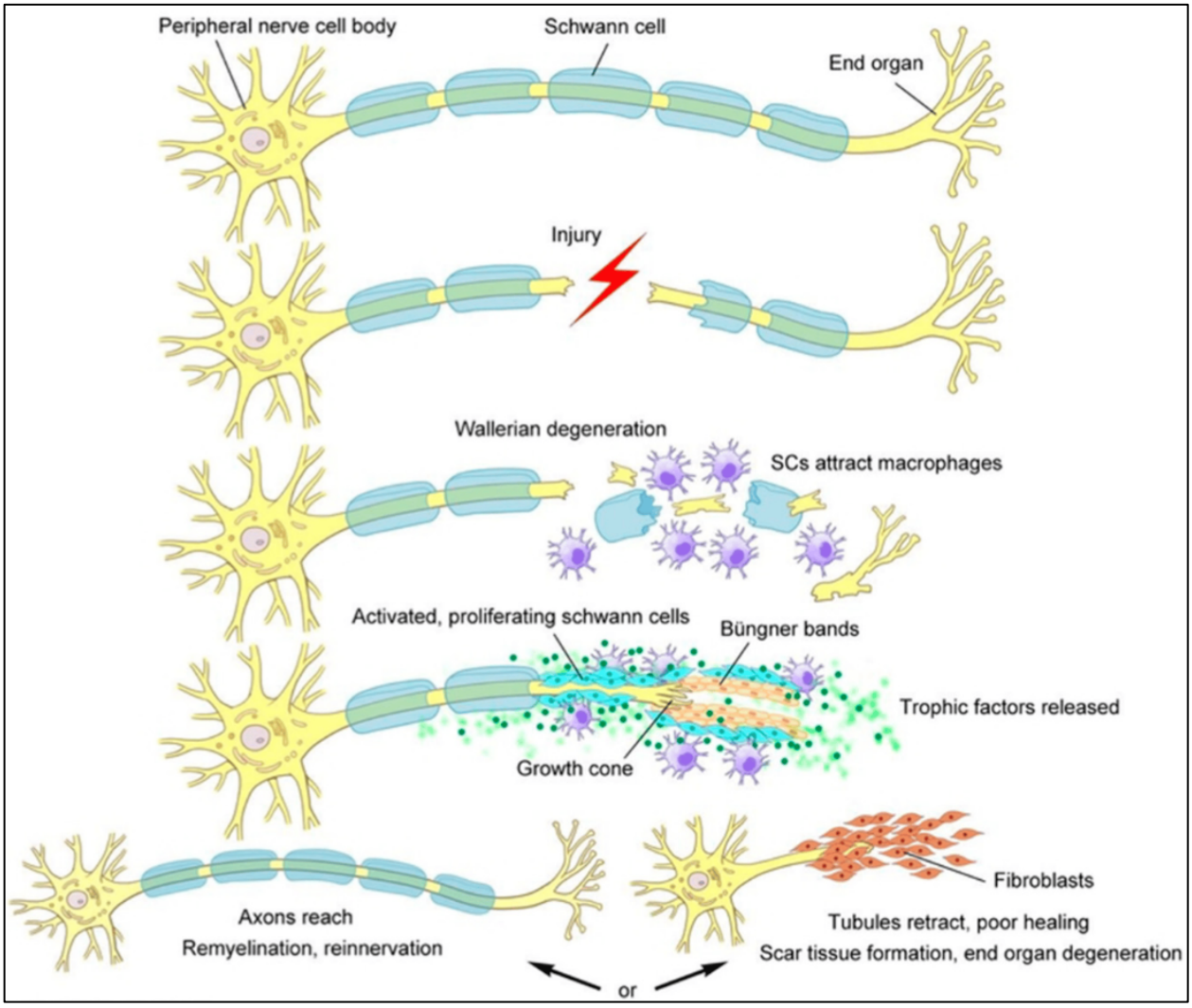

Figure 3: A schematic outline of a peripheral nerve cell undergoing WD. Following injury, activated SCs and macrophages are recruited to phagocytose the axonal debris. SCs are then stimulated to divide and form extensions over ECM molecules, enabling them to form bands of Büngner, which aids in guiding the growth cone across the injury gap. Extended denervation can result in scar tissue formation, preventing end-organ reinnervation and functional recovery (Juckett et al., 2022).

6 Current methods, their pitfalls, and where research has shifted now

Peripheral nerve axons regenerate at a rate of 1 to 5 mm/day (Gu et al., 2011; Nagappan et al., 2020; Sulaiman & Gordon, 2013). While this is rather remarkable, considering the microscopic nature of these cells, it remains much too slow for macroscale injuries, which can take months (or even years) to re-establish themselves (Sulaiman & Gordon, 2013). Quite generally, in assessing regenerative capacities of peripheral nerves, the size of the injury, as well as the species

of the injured organism (e.g., humans, mice, etc.), needs to be considered as they both limit the regeneration across the nerve gap (Contreras et al., 2023). Experiments on regenerating axons have demonstrated that they are able to reinnervate themselves through empty synthetic conduits for gaps ranging up to 4 mm in mice (Buti et al., 1996), 10 mm in rats (Lundborg et al., 1982); and 30 mm in primates (Archibald et al., 1995). Larger gaps are mostly unsuccessful at being bridged, meaning that repair attempts generally do not result in functional recovery (Contreras et al., 2023). PNIs of larger calibre (e.g., 20 mm or larger in humans) require external aid to successfully bridge gaps, as well as supporting the cells involved in regeneration and reinnervation (Lackington et al., 2017).

Initially, external interventions focused on autografts, allografts, and xenografts (Sarker et al., 2018). As a result, the current "gold standard" procedure for reconstructing such gaps are autologous nerve grafts, as they can successfully bridge the largest range of lesions. For this approach, donor grafts are collected, often from the patient's sural nerve and subsequently transplanted into the site of the PNI (Lackington et al., 2017). The main objective of the graft is to guide the regenerating axons in the direction of the distal nerve stump to allow end-organ reinnervation. Although this approach has been successfully used, for example, to repair digital nerve injuries ranging from 15 to 60 mm (via a 4.3 two-point sensory discrimination test) (Wang et al., 1996), there are numerous limitations. Autologous nerve grafts suffer from the requirement of invasive surgeries, making them susceptible to immunological rejection, risk of infection, and permanent damage or loss of function at the donor site. There is also limited availability of graft tissue, should larger quantities be required in more severe lacerations (Lackington et al., 2017). Additionally, SCs in the graft can undergo necrosis during transplantation partially due to poor profusion in the absence of the surrounding vasculature. Often, this occurs because of incompatible graft sizes for the injury (Lackington et al., 2017). For example, when the graft is too thick, revascularization is spatially limited and may not reach the graft's center. For grafts that are too large, the encompassing vasculature is not able to supply sufficient nutrients and oxygen to the entire structure, similarly leading to necrosis and non-functional repair (Lackington et al., 2017).

Motivated in part by these limitations, tissue engineering has surfaced as a viable alternative to autologous nerve grafts (Marquardt & Sakiyama-Elbert, 2013). Researchers can utilize biomaterials to engineer NGCs that can bridge more severe PNIs without the need for donor tissue and extra surgical procedures. NGCs are a form of tissue engineering and to date, there are eleven Food and Drug Administration (FDA) approved commercially available NGCs (e.g., Neurotube™ (K983007, 1999), Salubridge™ (K002098, 2000), NeuraGen™ (K011168, 2001) etc.) (Stocco et al., 2023). Generally, NGCs are constructed from either natural, synthetic, or semisynthetic biomaterials. The FDA approved conduits consist of a variety of materials including; non-biodegradable synthetic polymer (polyvinyl alcohol), biodegradable synthetic polymers (poly(DL-lactide-ε-caprolactone); polyglycolic acid), and biodegradable natural polymers (collagen type I, both with and without glycosaminoglycan; chitosan; or porcine small intestinal submucosa) (Stocco et al., 2023). As alluded to earlier, NGCs are predominantly cylindrical constructs that have either a hollow or filled luminal space with a supporting internal structure. Such supporting structures (e.g., hydrogels, fibers, etc.) are typically composed of biomaterials

and function to aid in both bridging the injury gap, while also acting as guiding tracks for regenerating axons through the NGC towards their target (Kehoe et al., 2012; Lackington et al., 2017; Sarker et al., 2018). FDA data has shown that collagen, chitosan, and poly (DL-lactide-ε-caprolactone) are both the most frequently used and approved materials for reconstructing peripheral nerves upon injury (Kornfeld et al., 2019). Among other factors, this is likely attributed to higher preservation of natural physical and biochemical cues contributing to axonal regeneration and interim PNR (Sarker et al., 2018).

6.1 Neural guidance conduit design

Creating a microscopic "tube" with internal scaffolding, sourced from bioavailable materials, for the explicit purpose of guiding nerve regrown – a conceptually simple concept – is no easy task! Initially, when guidance conduits gained popularity, focus was placed on the use of hollow conduits to recapitulating the endoneurium of the nerve to facilitate bridging (Sarker et al., 2018). Although the concept showed some promise, it quickly became evident that solely using conduits without any inner components was inadequate. In particular, these structures failed to modulate the physical and chemical cues necessary for complete neural regeneration, especially in severe injuries (Sarker et al., 2018). As mentioned earlier, by virtue that neural responses are highly sensitive to their environment, regeneration in such simple conduits is generally limited to only short distances (e.g., < 20 mm in humans) (Kehoe et al., 2012; Lackington et al., 2017; Sarker et al., 2018). Consistent with this finding, both clinical and animal model studies using individual hollow NGCs for cases of $\leq$ 30 mm nerve lesions resulted in axon receptor (Marquardt & Sakiyama-Elbert, 2013; Sarker et al., 2018). This phenomenon is then amplified through axonal scattering (i.e., axons grow radially without directionality), leading to non-functional and disoriented axons. Thus, hollow constructs served as an important proof-of-concept in the creation of functional guidance conduits but emphasized the need for more complex design characteristics.

One of the key design characteristics of NGCs is the permeability of the outer conduit walls, which facilitate their interaction with the surrounding environment. Specifically, permeability of the outer membrane dictates the inter-diffusion of nutrients, growth factors, waste products, revascularization, and radial cell infiltration (and/or extrusion) (Lackington et al., 2017). Porous tube walls (~50 μm pores), such as those made by lithography for example, allow these constituents to freely enter and exit the conduit. Crucially, they allow for SCs to migrate into the conduit, enabling their proliferation and axonal guidance within the conduit. On the other hand, they also allow for the entry of fibroblasts, which can deposit proteoglycans (e.g., chondroitin sulfate) that are known to impede neural regeneration (Sarker et al., 2018; Soller et al., 2012).

Contrary to permeable constructs, nonporous or impermeable conduit walls impede the exchange of substances with the surrounding environment. Due to the dynamic and growing environment, an accumulation of substance (e.g., extracellular fluid) can result in nerve compression (Sarker et al., 2018), which is undesirable. Semipermeable NGCs also exist, serving as an intermediate construct design. These function similarly to permeable conduits in that they

allow for substance exchange; however, their generally smaller pore sizes (~10 μm) allow them to selectively exclude certain cells (e.g., fibroblasts, scar tissue forming cells, etc.) from entering the conduit (Kehoe et al., 2012; Lackington et al., 2017; Sarker et al., 2018).

Over the years, researchers have transitioned from mostly working with hollow constructs with limited permeability towards filled constructs with semipermeable walls, as they provide better control over the exchange of factors around the injury. Although filled and semipermeable NGCs have shown some success, they have not yet fully replaced autografts in the treatment of PNIs, especially for lengths > 20 mm. However, through experimenting with different designs, key factors that promote regeneration and reinnervation of the nerve have become more apparent and include but are not limited to permeability, microenvironment (cellular and acellular), microarchitecture, topographical design, mechanical properties, and molecular microenvironment (Gonzalez-Perez et al., 2018; Marquardt & Sakiyama-Elbert, 2013). A large body of research exists surrounding the use of the native ECM molecules to address these design requirements.

7 Role of ECM in PNS regeneration

The ECM is a non-cellular physiologically interactive matrix composed of proteins and carbohydrates. Its three-dimensional network is found beyond the immediate vicinity of the plasma membrane (i.e., intercellular space) throughout all tissues, where it is involved in several cellular processes (e.g., migration, proliferation, and differentiation). Additionally, it contributes structural support and aids in balancing intercellular signalling. Constituents of the ECM bind one another in addition to cell adhesion receptors (e.g., integrins, cadherins etc.), constructing an environment for cells and tissues to occupy (Gonzalez-Perez et al., 2013).

Several ECM molecules can be directly linked to integral roles executed in the developmental stages of the nervous system. For instance, laminin, the main protein involved in the maturation process of the PNS, has been shown to be vital for SCs to successfully myelinate axons (Gonzalez-Perez et al., 2013, 2018). Similarly, fibronectin, a non-collagenous glycoprotein is involved in the migration and differentiation of neural crest cells during neurulation (Gonzalez-Perez et al., 2013, 2018). In fact, fibronectin knockouts are embryonically lethal *in vivo* (Paten et al., 2019). To this end, considering that ECM molecules play such crucial roles in the developmental stages of the nervous system, it is likely that they are also important for regeneration.

7.1 ECM influences cell behaviour on scaffold

For axons to grow, they are required to adhere and attach to surrounding surfaces, allowing them to sprawl and extend (Figure 2). In order for cells to interact with ECM molecules, cell adhesion molecules are required, as they facilitate the binding process. There are numerous kinds of cell adhesion molecules; however, those most essential for ECM molecules (e.g., Laminin and Fibronectin) are integrins (Gonzalez-Perez et al., 2013). The expression of integrins on growth

cones enable elongating axons to interact with their surrounding ECM (Figure 4). There are numerous subunits within different integrin families; these allow certain integrins to specifically interact with ECM molecules (Gonzalez-Perez et al., 2013). This showcases that although these integrins garner us the ability to target specific ECM constituents, utilizing the cells inherent expression of multiple integrin types to bind a wide array of ECM molecules may hold the key to successful PNR.

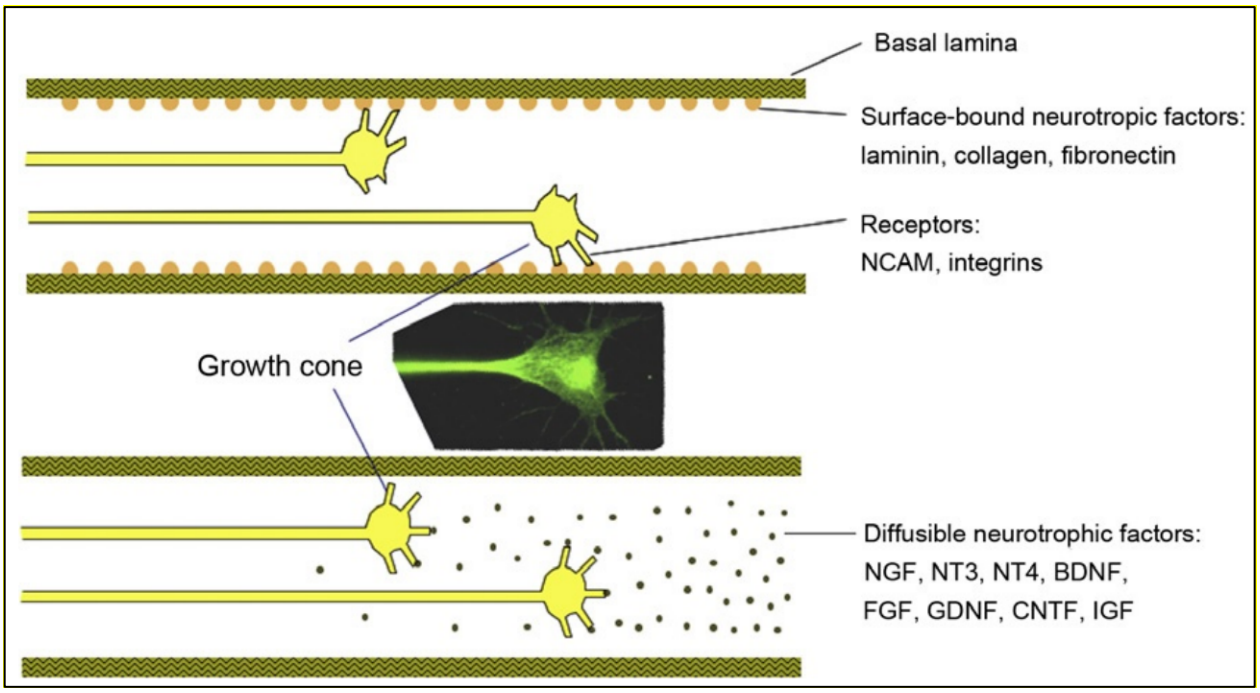

Figure 4: Sketch of the guidance cues that extending growth cones receive during axonal elongation. These molecular cues include ECM components such as: laminin, fibronectin, and collagen (Gonzalez-Perez et al., 2013).

Laminin is a key component in peripheral nerve ECM, contributing to axonal growth both *in vivo* and *in vitro*. Laminin is found continuously throughout the basement membrane, as well as in the endoneurium and perineurium of peripheral nerves (Gao et al., 2013). Fibronectin, on the other hand, functions in mediating cell-binding (Gonzalez-Perez et al., 2013). Both constituents are, however, synthesized and secreted by SCs. Interestingly, ECM molecules are crucial to SCs forming Bands of Brünger, although the exact mechanism is not fully understood. Bands of Bünger SCs are essential in guiding regrowing axons and their ability to migrate is strongly correlated to axonal grow. Transwell migration assay results found that both fibronectin and laminin promote SC migration, whereby laminin seems to have more significant effects (Yu et al., 2023). This positive feedback loop between SCs depositing ECM components that are then required for axonal guidance and growth demonstrates the dependence of various constituents on one another. This helps explain why the most successful nerve regenerations were performed by emulating the natural environment of the nerve and why autologous nerve grafts are the current preferred method of PNR given their endogenous nature.

7.2 Collagen type 1 as a scaffold in NGCs

The addition of ECM molecules and cells into these constructs has been found to both support and enhance axonal regeneration (Gonzalez-Perez et al., 2018). Currently, there are several different methods used to fill NGCs (e.g., hydrogels and fibers with and/or without cells). Here, we shall focus on exploring collagen, as it has become one of the most widely used – and successfully implemented – biomimetic biomaterials for PNR. In general, such biomimetic materials aim to emulate the native microarchitecture of the nerve by ensuring the composition and shape provide similar mechanical properties to peripheral nerves in their native ECM.

Collagen is the most abundant protein in mammals and plays integral roles in the structural maintenance of ECM in several tissues, including peripheral nerves (Ahmed et al., 2021). Within vertebrate genomes, there are 28 known types of collagens, each differing in structure, size, and function (Naomi et al., 2021). Within the PNS, two classes of collagen molecules are expressed: fibril forming collagens (type I, III and V) and basement membrane collagens (type IV) (Naomi et al., 2021). Of these, type I collagen is most abundant, accounting for around 90% of proteins in the human body (Naomi et al., 2021). In peripheral nerves, type I collagen aids in maintaining the basement membrane, a structure both synthesized and regulated by SCs. As a vital structural and connective tissue, the basement membrane is essential in both supporting SCs and regenerating axons. As such, collagen's mimicry of healthy, native structural components of peripheral nerves makes it ideal for this task (Koopmans et al., 2009; Lackington et al., 2017).

As alluded to above, the rate of successful PNR, independent of the material used, is inversely proportional to the size of the severed nerve and collagen-based NGCs are no exception to this "rule." For example, in one noteworthy success story, a collagen-based luminal filler within a polyglycolic acid outer tube was used to bridge the gap of a severed 5 mm rat peroneal nerve, yielding PNRs analogous to autografts (Rosen et al., 1990). By contrast, another study using collagen nerve guides with a polymer mesh (consisting of Kevlar fibers) failed to bridge an 18 mm gap in the sciatic nerve of a rat (Ansselin et al., 1997). Generally, early studies like this (as well as many thereafter) highlight that collagen-based constructs aid in bridging PNIs (Li et al., 2014); however, they showed a lacking ability to repair larger gaps – a challenge that remains prominent today.

8 Conclusion

In summary, PNIs account for a significant fraction of trauma injuries found throughout society resulting in a range of physical, psychological, and socio-economic complications. Although the current preferred method of treatment, the autograft, is successful in bridging short lesions, it leaves much to be desired and is unsuccessful in bridging longer gaps (i.e., > 20 mm). To address these limitations, the use of NGCs is a promising contemporary alternative. Consisting of a cylindrical shape with an internal scaffolding, NGCs can control the influx and efflux of various constituents (e.g., waste, nutrients, and cells), depending on their permeability and pore size. In

particular, the incorporation of ECM molecules within the conduits has been shown to influence the behaviour of neural cells, enabling them to be guided during regeneration. To date, although NGCs have not yet surpassed autografts in regenerative performance, they show potential as regenerative aids, given their flexible design characteristics and ability to imitate the native environment of regenerating peripheral nerves. In particular, the use of biodegradable materials to design NGCs that aim to mimic the native environment of nerves provides an exciting avenue within the field of tissue engineering to further improve functional recovery of PNIs. To realize this goal, further research focusing on better understanding processes contributing to PNR will be essential in finding more effective methods to treat PNIs.

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