Biomimetic Architectures for Peripheral Nerve Repair:
A Review of Biofabrication Strategies

Paul A. Wieringa, Ana Rita Gonçalves de Pinho, Silvestro Micera, Richard J. A. van Wezel, and Lorenzo Moroni*

Biofabrication techniques have endeavored to improve the regeneration of the peripheral nervous system (PNS), but nothing has surpassed the performance of current clinical practices. However, these current approaches have intrinsic limitations that compromise patient care. The “gold standard” autograft provides the best outcomes but requires suitable donor material, while implantable hollow nerve guide conduits (NGCs) can only repair small nerve defects. This review places emphasis on approaches that create structural cues within a hollow NGC lumen in order to match or exceed the regenerative performance of the autograft. An overview of the PNS and nerve regeneration is provided. This is followed by an assessment of reported devices, divided into three major categories: isotropic hydrogel fillers, acting as unstructured interluminal support for regenerating nerves; fibrous interluminal fillers, presenting neurites with topographical guidance within the lumen; and patterned interluminal scaffolds, providing 3D support for nerve growth via structures that mimic native PNS tissue. Also presented is a critical framework to evaluate the impact of reported outcomes. While a universal and versatile nerve repair strategy remains elusive, outlined here is a roadmap of past, present, and emerging fabrication techniques to inform and motivate new developments in the field of peripheral nerve regeneration.

1. Introduction

The use of tissue-engineered scaffolds to improve or control the regeneration of the peripheral nervous system (PNS) has been an area of extensive research for the past 30 years. The PNS has an intrinsic capacity to regenerate, such that minor damage can often be repaired spontaneously. However, if the damage is too extensive, with major disruption of the native extracellular matrix (ECM) structure, functional recovery becomes severely compromised or impossible.[1] The clinical “golden standard” for repairing such PNS injuries is the insertion of autograft (a nerve segment harvested from the patient) within the gap between the transected ends of a severed nerve. While this provides a similar ECM environment conducive to regeneration, it also requires additional surgery to harvest a nerve segment from an underutilized region of the patient which can lead to tissue morbidity at this donor site.[2,3] To circumvent the use of an autograft, alternative tissue engineering strategies have been developed to improve regeneration to optimal levels typically observed in minor cases. This has prompted the development of tissue-engineered synthetic scaffolds in treating nerve injury.

The nervous system of the human body is traditionally separated into the central nervous system (CNS), comprised of the brain and spinal cord, and the PNS, comprised of a network of neurons that connect the CNS to the body. In most basic
terms, the CNS is responsible for processing and integrating incoming information, decision making, and, subsequently, initiating actions. Meanwhile, the PNS is comprised of afferent neurons, which relay sensory information from the body back to the CNS (afferent signals), and efferent neurons, which transmit signals from the CNS to limbs and organs (afferent signals).

Although it is not the focus of this work, CNS regeneration is recognized as a related area of research in the development of repair strategies; many recent reviews describe advances for the restoration of brain function after disease or trauma as well as repair after spinal cord injury (SCI).[^6[^17]](footnote)

SCI repair, in particular, has garnered much interest as an example of the CNS with a lower capacity for regeneration compared to the PNS. This is attributed primarily to the postinjury glial scar formation at the site of SCI, creating an inhibitory microenvironment of ECM components that physically and chemically prevent the successful regeneration of CNS neurons. The complex array of biological signals involved still have to be fully explored and, as such, the therapeutic application of tissue engineering solutions has had limited success.

In contrast, the PNS exhibits a higher regenerative capacity and has been more amenable to therapeutic intervention. Scaffold structures that bridge the gap of a transected nerve can enable recovery from otherwise debilitating PNS injury; basic tube-like nerve guidance conduits (NGCs) are able to repair 10 mm defects and represent a clinically successful alternative to the autograft.[^18] However, despite the encouraging results of NGCs, there are still limitations to the extent of successful recovery from PNS injury; the reader is directed to the work of Daly et al.,[^19] Arslantunali et al.,[^20] and Gaudin et al.[^21] for thorough reviews of NGCs. Whereas an NGC is essentially a hollow lumen, the ECM of both the natural PNS environment and the autograft exhibits a mesoarchitecture and microenvironments conducive to guided neurite regeneration, which are far beyond the currently available NGCs. Recent developments aim to produce scaffolds that emulate aspects of the native ECM, presenting appropriate biochemical and physical cues in a spatiotemporally relevant manner to improve cell response.

Here, we describe tissue engineering strategies for optimized PNS regeneration, focusing, in particular, on recent developments in the design and fabrication of physical cues and structural elements of interluminal guidance scaffolds. After establishing the parameters of an ideal synthetic ECM, current strategies are discussed according to three main categories: (i) isotropic hydrogel fillers, supporting cell growth via intrinsic material properties to promote nerve repair; (ii) fibrous interluminal fillers, comprised of oriented fibrous elements that provide additional structural support within the lumen but without hierarchical structure; and (iii) patterned interluminal scaffolds, designed with a higher degree of complex organization to reflect the PNS ECM. A brief mention of other promising design elements is provided, such as the incorporation of cell adhesion peptides, diffusible growth factors, and support cells which produce both growth factors and ECM molecules. This is followed by a prospective on future design considerations and potential applications.

---

[^6][^17]: References for SCI repair and CNS regeneration.

[^18]: Despite the encouraging results of NGCs, there are still limitations.

[^19]: Reference for the work of Daly et al.

[^20]: Reference for the work of Arslantunali et al.

[^21]: Reference for the work of Gaudin et al.
supported by a collagenous “endoneurium.” Each fascicle is delineated by a “perineurium” sheath, a perineural epithelial cell layer serving as a blood–nerve barrier, and individual fascicles are bound into one nerve trunk by the “epineurium” and the outermost “mesoneurium.”[18,22] The immediate endoneurial environment of these axons is comprised of both type I and type III collagen fibrils,[23,24] as well as a basal lamina of collagen type IV, fibronectin, and laminin molecules.[25] These ECM components are organized to surround the axons to form endoneurial tubes, characterized by aligned collagen fibers produced by myelinating SCs.[26,27] Tube dimension correlates to the size of resident axons, ranging in diameter from 2 to 20 µm.[28] The interstitial spaces between endoneurial tubes are filled with hydroscopic glycosaminoglycans and also contain fibroblasts.[29,30] Macroscopically, endoneurial tubes are homogeneously interspersed within fascicles and the residing axons occupy ≈50% of the overall cross-sectional area of the endoneurium.[31,32]

2.2. Neural Regeneration

Functional recovery from peripheral nerve injuries (PNI) remains a significant clinical challenge,[34,35] mainly because of the complexity of the nervous system’s anatomy and physiology.[36,37] Damage to the nervous system, caused by mechanical, thermal, chemical, or ischemic factors, can disrupt the axonal connection between neural cell bodies and innervated tissue and impair various functions.[37] PNI most commonly results from blunt trauma (nerve crush) and soft tissue disruption from either laceration or elongation associated with fractures and fracture dislocations.[18,38,39]

A PNI, where the nerve trunk is severed, results in a proximal nerve stump, the still viable end of the nerve fiber connected to neural cell bodies, and the distal nerve stump, on the side of the innervation targets.[37] The distal stump undergoes Wallerian degeneration that begins with the removal of debris, including the distal segments of the severed axons that are effectively dead. This process includes the disintegration of axoplasmic microtubules and neurofilaments,[40] the activation of macrophage migration into the degenerating nerve stumps, and the phagocytose, the disintegrating nerve fibers, and myelin.[41] Once free of debris, the intact distal endoneurial tubes provide an ideal substrate for SCs to proliferate and form the bands of Büngner, oriented longitudinal bands of SCs that help in the process of regeneration.[42,43] Furthermore, these distal SCs synthesize molecules and diffusible growth factors that enhance neurite growth.[44]

On the proximal end, “regenerating units” are formed by both myelinated and unmyelinated fibers.[45] This involves the sprouting of a growth cone from the damaged axon, an actin-based structure that forms to sense the immediate environment in order to steer the regenerating axon as it elongates to its target.[46] Growth cones are guided to their targets by a combination of contact-mediated (haptotactic) and diffusible (chemotactic) cues.[46]

As growth cones explore, membrane-bound integrins attempt to bind adhesion sites on the surrounding ECM proteins (i.e., collagen, laminin, and fibronectin), forming focal adhesion (FA) complexes upon which the growth cone can pull. The strength of an FA can be modulated by substrate topography and stiffness[47–50] as well as diffusible cues within the microenvironment.[51] Only a sufficiently strong FA persists, dictating the direction and speed of growth cone advance. Similarly, SCs also rely on FA formation for migration and proliferation.[52,53] In this sense, directed neurite growth and nerve repair depend on whether the microenvironment promotes successful modulation of FA formation.

The endoneurial tubes are critical in this respect, presenting cells with both adhesive and topographical cues in the form of laminins and oriented collagen fibrils. Furthermore, the characteristic 3D microchannel architecture provides the structural support needed for SCs to form the growth-promoting bands of Büngner and also serves to corral neurite growth in the direction of the target tissue for efficient nerve repair.[43] Rapid neurite growth is essential because the presence of viable SCs in the distal nerve segment and the overall timing of the repair is critical to recovery; the regenerative SC phenotype is lost after 1–2 months post injury[54–56] and axons that fail to reach the target tissue by this point are withdrawn or “pruned,” resulting in reduced nerve function.[37]

While some have reported that small nerve gaps (<5 mm) can spontaneously heal,[1] this likely occurs with diminished functional recovery and some form of surgical intervention is typically required.[57] To determine the required course of action, a widely used grading system currently used to assess the degree of damage, and potential success of peripheral nerve repair was developed by Seddon[58] and Sunderland[59] and expanded by Mackinnon[60] (shown in Table 1).[58,59,61]

2.3. Autografts and Allografts

Owing to the profound impact of PNS damage, many strategies have been developed and others are being investigated to facilitate axonal reinnervation and to direct axonal outgrowth.[66] When proximal and distal nerve segments are unable to be joined without tension, the resulting gap is typically replaced with a segment of nerve from a less important site of the same patient, typically the sural nerve (Figure 2).[62] Despite advances,
no other approach has achieved superior performance, leaving autografts to remain the clinical gold standard in bridging nerve injury gaps.\(^\text{(37)}\) This is attributed to the presence of optimal structural cues and the presence of viable Schwann cells, which concomitantly offer excellent support for oriented axon regeneration.\(^\text{(63)}\)

However, nerve autografting has inherent flaws: a second surgery is required to retrieve the donor tissue;\(^\text{(18)}\) possible donor site morbidity or painful neuroma formation; the length and/or diameter of the autologous nerve may be insufficient for effective reconstruction.\(^\text{(65)}\) Another major shortcoming of this technique is the fact that endoneurial tubes can never be exactly reapproximated, resulting in a mismatch of regenerated axons. This leads to inappropriate and incomplete reinnervation, and subsequent poor recovery in function.\(^\text{(66)}\) Finally, autograft use can be limited due to availability of donor material.\(^\text{(67)}\) For more extensive repair or gaps larger than 5 cm, the use of an allograft is required due to the reasons mentioned above.\(^\text{(68-70)}\)

An allograft is a decellularized nerve segment which has been harvested from a cadaver. Decellularization is a process that removes or destructs the cells present in a tissue, preventing the strong immunogenic response that implantation of the tissue would cause in the host while still preserving the structural elements (ECM components) from the donor. In this way, the 3D structure and composition of the native nerves are maintained.\(^\text{(71)}\) Several studies have revealed that the further removal of axonal-growth-inhibitory molecules (e.g., chondroitin sulfate) from the basal lamina could potentially increase the regenerative efficacy of acellular allogeneic nerve grafts.\(^\text{(72,73)}\) While cadaveric nerve allografts can be rejected by the host and require adequate immunosuppression, recent studies have shown that thorough removal of cellular debris can limit the immune response while permitting nerve regeneration.\(^\text{(75,76)}\)

The resulting cell-free tissues can be used as an alternative to autologous nerve grafts, especially in the repair of noncritical peripheral nerve gaps with a small length and diameter.\(^\text{(74,75,77)}\) Decellularized allografts have been used in the clinic for the repair of facial nerve defects in humans and an allograft product approved by the food and drug administration (FDA) is now available (AxoGen).\(^\text{(78)}\) A number of recent studies and reviews highlight the potential clinical application of this approach for defects ranging from 3 to 7 cm or more.\(^\text{(67,68,79,80)}\) However, results are also inconsistent, and allografts are subjected to many of the same issues of the autograft, particularly size mismatch and availability of donor material.

### 2.4. Ideal Nerve Repair and Current Limitations

In response to the limitations of autografts and allografts, research has focused on developing synthetic alternatives. This work falls under the purview of tissue engineering, an interdisciplinary field that applies the principles of engineering and life-sciences to create bioactive scaffolds that are capable of restoring, maintaining, or improving tissue function.\(^\text{(81)}\)

Ultimately, a synthetic nerve guide should exhibit performance that surpasses the autologous nerve graft in order to be considered clinically viable and relevant. Today, the use of hollow NGCs is a clinically approved alternative to autograft repair.\(^\text{(66)}\) These hollow tubes are used to bridge a nerve defect gap, with the proximal and distal ends of a transected nerve fixed into the respective ends of the tube. For small “subcritical” injury gaps (around 3–10 mm), NGCs have shown successful clinical results to the level of the autograft.\(^\text{(18)}\) Improving nerve fascicle alignment and obviating the need of a second surgery.\(^\text{(37,40)}\)

When an entubulation strategy is applied after PNI, a protein gel forms within the NGC from endogeneous proteins produced by the transected ends of the nerve.\(^\text{(80)}\) A major component of the ECM that fills the NGC at this initial stage is fibrin, a wound-healing associated protein that contains cell adhesion motifs.\(^\text{(83,84)}\) Provided that the nerve gap is not beyond the critical length, a fibrin cable forms within the NGC and neurite regeneration occurs.

| Sunderland | Seddon | Injury | Neurosensory impairment | Recovery potential |
|-----------|--------|-------|-------------------------|-------------------|
| I         | Neuropraxia | Intraneuronal edema, conduction block | Neuritis, paresthesia | Full (1 d to 1 week) |
|           |         | Possible segmental demyelination | Neuritis, paresthesia | Full (1–2 months) |
| II        | Axon severed, endoneurial tube intact | Paresthesia, episodic dysesthesia | Full (2–4 months) |
| III       | Axonotmesis | Endoneurial tube torn | Paresthesia, dysesthesia | Slow, incomplete (12 months) |
| IV        | Only epineurium intact | Hypoesthesia, dysesthesia, and neuroma formation | Neuroma in continuity |
| V         | Neurotmesis | Loss of continuity | Anesthetic, intractable pain, and neuroma formation | None |
| VI        | Combination of above | Combination of above | Unpredictable |

![Figure 2. Schematic representation of an autologous nerve graft. The alignment of the fascicles is critical for successful regeneration of peripheral nerves. Reproduced with permission.\(^\text{(64)}\) Copyright 2013, Elsevier.](image-url)
through this matrix.\[85\] Both neuronal and non-neuronal cells are known to invade and remodel this environment, depositing other constituent ECM proteins such as collagen and laminin.\[86,87\]

Because they present a more controlled and enclosed environment,\[34\] the penetration of fibrous scar tissue into the neural defect is minimized and, conversely, there is a local accumulation of growth factors produced by the nerve stumps that help the process of regeneration.\[42\] At the same time, NGC porosity is also important for sufficient nutrient delivery.\[67\]

Recent NGC development has focused on new fabrication methods to enhance the mechanical properties and porosity of the luminal wall and the use of different materials, both natural and synthetic.\[19,20,57,63,88,89\]

However, the efficacy of hollow NGCs is currently limited to a critical nerve gap, a species-dependent value ranging from 5 mm in mice to ≈3 cm in primates (see Figure 3 and Table 2).\[90\] Beyond this size, deficient levels of regeneration have been observed. This is usually attributed to an inadequate formation of ECM components during the initial stages of regeneration which fail to create a fibrin cable structure.\[91,92\] This limits the migration of native SCs into the site of the lesion and reduces in the formation of glial bands of Büngner.\[92–94\]

To increase the critical gap length a synthetic NGC can bridge, considerable efforts have been made to overcome this failure of ECM self-assembly by developing NGCs with internal lumen architecture. This includes the incorporation of topographical cues, based on in vitro studies that show neurite growth is directed by FA orientation in response to underlying aligned topographies.\[100–102\] In addition, studies have also shown that microchannel structures can confine neurites and serve to effectively direct nerve growth.\[103–105\] Furthermore, favorable cell–material interaction and cell adhesion are paramount to establishing sufficient neurite growth in the first place. This can be achieved by using native ECM proteins that intrinsically support cell adhesion and nerve growth or, alternatively, selecting synthetic materials that display cell adhesion motifs on their surface, either through chemical grafting\[106,107\] or aspecific adsorption of endogenous proteins onto the surface.\[108–110\] Therefore, strategies to improve nerve repair often incorporate one or more of these aspects in order to recapitulate, to a lesser or greater extent, the regeneration capacity provided by the native PNS ECM.\[92\] As this work has evolved, a number of ideal design requirements have emerged as guiding principles for continued nerve graft development. An ideal nerve repair device should have the following characteristics:\[34,40,62,66,90,91,111–115\]

(a) direct axon growth from the proximal to the distal nerve stump;
(b) have porosity between 5 and 30 µm (ideally, 10–20 µm) in order to limit the infiltration of fibrous scar tissue while allowing diffusion of nutrients into the conduit and waste products to exit the conduit;
(c) present appropriate mechanical properties throughout the regenerative process, with sufficient flexibility to avoid compression of neural tissue while still providing structural support for the regeneration of nerve fibers as well as any sutures required for immobilization;
(d) create an optimal microenvironment for nerve regeneration and promote suitable cell–surface interactions;
(e) be made from biocompatible materials that are nontoxic to cells, elicit little, or no immune response, and may even enhance cell behavior for improved regeneration;
(f) be made from materials with suitable biodegradability, such that the device is naturally adsorbed by the body, and a second surgery to remove the device is not required;
(g) maintain a suitable shelf life, amenability to sterilization techniques, and exhibit an overall low immune response.

This review focuses on techniques used in the fabrication of structured interlumen fillers, beginning with an overview of design considerations and current strategies, followed by an assessment of emerging approaches and promising methods (summarized in Table 3) that may have potential in creating an ideal, synthetic neuroregenerative scaffold.

### 2.5. Assessing Nerve Regeneration: A Discretionary Note

Potentially subtle discrepancies between studies can make it difficult to draw comparison between different strategies for PNI repair. As mentioned in the previous section, the gap length is an extremely important factor when evaluating nerve repair and is intrinsically species dependent. The most frequent animal models remain mouse and rat, although Kaplan

---

**Table 2. Critical size defects.**

| Animal | Nerve  | Defect size [cm] | Type of NGC                  | Reference |
|--------|--------|------------------|------------------------------|-----------|
| Human  | Digital| 3                | PCA\(^*\) tube               | [95]      |
| Monkey | Ulnar  | 3                | Pseudosynovial tube PCA\(^A\) tube | [96,97]  |
| Rabbit | Peroneal | 3            | Vein conduit                 | [98]      |
| Rat    | Sciatic | 1                | Silicone conduit             | [99]      |
| Mouse  | Sciatic | 0.5              | Silicone conduit             | [36]      |

\(^A\)PGA - poly(glycolic acid)
While studies involving defects of a critical size or smaller (subcritical) can still be informative, caution must be exercised when interpreting the significance of resulting stemming from these studies. Evaluation of studies performed with a defect size at or below the critical length should ideally compare the quality and speed of the regenerative process with respect to the already robust response of an NGC. However, methods to assess peripheral nerve regeneration are also not uniformly applied, again impeding one’s ability to make direct comparisons. The most common methods are outlined by Vleggeert–Lamkamp, where it is also stressed that a proper evaluation of neuroregenerative capacity.

A strong neuroregenerative response. To assess functional recovery, measurements of the velocity and amplitude of evoked compound muscle action potentials can be performed. Additionally, return of motive function can be measured by assessing the walking gait of recovering animals, while sensory recovery can be determined via the response to noxious stimuli (i.e., the “pinch test”). In discussing the reported results, the authors confined general PNI recovery to pertain specifically to histological assessment. The assessment of electrophysiological function or motive function, if performed, is otherwise explicitly described.

### 3. Isotropic Hydrogel Fillers

Hydrogels are attractive biomaterials for nerve regeneration mainly because of their physical properties that are similar to native tissues, allowing the creation of a 3D environment that better mimics the biological properties. Hydrogels have the ability to swell in water and retain a significant fraction of water within their structure, mimicking the hydrated ECM microenvironment. The mechanical environment with which cells may interact can affect their viability and behavior. Thus, the use of a hydrogel with specific mechanical and physical features, including stiffness, porosity, overall architecture, and degradability, can affect the function or subsistence of a hydrogel in a tissue (Figure 4).

Neurite outgrowth through dense hydrogels requires neurites to remodel the local hydrogel environment. For this to occur efficiently, sufficient cell adhesion and cleaving sites to facilitate matrix remodeling are required. Natural hydrogels based on endogenous proteins, such as fibrin and PNS ECM proteins, have the benefit that they often already incorporate natural cell adhesion sites and can also be remodeled by cells. In contrast, exogenous and synthetic hydrogels...
The fibrin protein is naturally excreted after injury [129] and can provide a suitable substrate for nerve repair, as described above. When gap defects are sufficiently small, the fibrin that accumulates within the NGC forms a cable that serves as a natural scaffold to facilitate nerve repair. Fibrin can also be easily isolated from the blood of a patient [130] providing a nonimmunogenic and cell-adherent scaffold material that has been employed in a number of tissue engineering applications [131–133]. As such, fibrin has been trialed as an interlumen material by prefilling the conduit with fibrin in lieu of endogenous filling. A series of earlier experiments by Williams found that presenting the regenerative nerve with such a gel-filled lumen had generally positive effects compared to an empty tube [134]. This was performed for only a 10 mm gap defect in rat sciatic nerve, just at the critical gap length for this animal model. It was observed from this work that performance was dependent on the gelation and composition of the fibrin matrix, with innervation increasing with lower matrix density and increased longitudinal arrangement of polymerized fibrin fibers; these factors were later confirmed in vitro [135,136]. Under these experimental conditions, a low-density fibrin matrix with sufficiently ordered fibers proved to be superior to an empty NGC. However, when evaluated for a gap length of 13 mm, neural outgrowth in fibrin-filled NGCs was comparable to that of hollow nerve guides. Yet, performance was markedly worse compared to the standard autograft [137].

3.1. Fibrin

The fibrin protein is naturally excreted after injury [129] and can provide a suitable substrate for nerve repair, as described above. When gap defects are sufficiently small, the fibrin that accumulates within the NGC forms a cable that serves as a natural scaffold to facilitate nerve repair. Fibrin can also be easily isolated from the blood of a patient [130] providing a nonimmunogenic and cell-adherent scaffold material that has been employed in a number of tissue engineering applications [131–133]. As such, fibrin has been trialed as an interlumen material by prefilling the conduit with fibrin in lieu of endogenous filling. A series of earlier experiments by Williams found that presenting the regenerative nerve with such a gel-filled lumen had generally positive effects compared to an empty tube [134]. This was performed for only a 10 mm gap defect in rat sciatic nerve, just at the critical gap length for this animal model. It was observed from this work that performance was dependent on the gelation and composition of the fibrin matrix, with innervation increasing with lower matrix density and increased longitudinal arrangement of polymerized fibrin fibers; these factors were later confirmed in vitro [135,136]. Under these experimental conditions, a low-density fibrin matrix with sufficiently ordered fibers proved to be superior to an empty NGC. However, when evaluated for a gap length of 13 mm, neural outgrowth in fibrin-filled NGCs was comparable to that of hollow nerve guides. Yet, performance was markedly worse compared to the standard autograft [137].

3.2. ECM Hydrogels

To further enhance the success of gel-based luminal fillers, different combinations of natural ECM proteins have also been trialed. Initial reports by Madison et al. describe the use of laminin/collagen-based fillers—an early, noncommercial form of Matrigel [138,139]. This was first applied to optic nerve regeneration in rabbits followed by a 5 mm PNI defect in mice [139]. Initial results showed improved regeneration over a 2 week period compared to a hollow tube [140] though a follow-up study revealed that long-term growth (6 weeks) was impeded [141]. Valentini et al. later confirmed this using different concentrations of either Matrigel and collagen-only gels for a 5 mm defect in the same animal model over a 12 week period [142]. The conclusion reached was that the degree of long-term regeneration was compromised by the presence of dense bulk luminal fillers, attributed to the physical impediment posed by the hydrogel itself. This early work depicts a scenario of neurite regeneration across gap lengths below the critical defect size where a solid hydrogel filler promotes initial neurite outgrowth, but inevitably impedes regeneration compared to the more permissive environment of a hollow tube.

However, this differs once gap lengths above the critical defect size are examined. Additional work examined neurite regeneration over a 16 week period, comparing Matrigel and collagen fillers to hollow NGCs for a supracritical defect gap of 15–20 mm in rats [143]. It was found that all tubes without filler experienced no growth, whereas tubes with a gel lumen (either Matrigel or collagen) experienced regeneration. This was later corroborated by a study that examined the effects of sub- and supracritical gap lengths in mice (4 or 6 mm), comparing regenerative growth over a 5 week period with lumen fillers consisting of various hydrogel compositions and densities [144]. For subcritical lengths, the lowest collagen and laminin concentrations performed best, surpassing the standard fibrin gel fillers, hyaluronate gel, and even the hollow NGC. This confirmed that presenting an ECM-like environment is beneficial for neurite regeneration, provided it is adequately permissive to innervation and cellular invasion. Supracritical lengths were then examined, focusing on diluted gel compositions. In this context, the laminin-containing Matrigel proved to be slightly better than collagen, reciprocating in vitro findings [145,146]. Despite these observations of modulated neurite growth by natural ECM hydrogel fillers, a comparison revealed that they were still outperformed by the “gold standard” autograft [147,148]. Furthermore, a more recent study also indicated that the presence of natural ECM bulk hydrogel can expedite the infiltration of non-neuronal cells into the conduit space, promoting fibrotic tissue formation and leading to impaired neural regeneration [149].

3.3. Exogenous and Synthetic Hydrogels

A number of other potential hydrogel sources are available which are not based on endogenous proteins. These include exogenous gels, such as agarose, alginate, or keratin, or synthetic hydrogels, such as those based on polyethylene glycol (PEG) [122,150–154]. Many of these materials are widely abundant, making them an attractive option compared to more scarce or...
expensive ECM proteins. Since these hydrogels do not exhibit cell adhesion proteins or other cell-signaling motifs, they also represent a class of biocompatible materials whose properties can be systematically tuned and controllably modified with specific bioactive molecules to selectively stimulate cell behavior.

However, these hydrogels have yet to find widespread application as NGC luminal fillers for in vivo PNS injury studies. Studies that have been performed support the conclusion that a less permissive environment presented by a bulk, dense hydrogel filler is not conducive to peripheral regeneration. The performances of agarose or alginate fillers over supracritical gap lengths have been shown to be equivalent or less, respectively, compared to a saline-filled NGC, requiring the incorporation of growth factors or additional adhesion proteins to improve regenerative performance. Even when applied, however, the performance of agarose fillers remains poor. In addition to hydrogel density, this difference can also be attributed to relatively poor cell adhesion compared to the native fibrin matrix.

Keratin is another exogenous protein used for creating a hydrogel luminal filler, recently shown to be a promising candidate for subcritical PNI defect size in mice. Long-term evaluation shows equivalent functional recovery and increased neurite growth compared to both a saline-filled NGC and an autograft implant, though application to a supracritical defect size is required to be able to fully assess the performance. Regardless, the availability of other promising hydrogel substrates, as well as the ability to design and specifically tailor synthetic materials for an intended application, holds great potential for the field of peripheral nerve repair and remains an exciting and developing area of research. Due to the limited scope of this class of hydrogels currently applied to PNI repair, readers are directed to relevant reviews for more general information.

3.4. Anisotropic Hydrogels: The Role of Hydrogel Fibril Alignment

Based on observations of improved neurite growth resulting from longitudinal fibril formation in fibrin gels, aligned fibrils were induced within collagen gels and laminin gels (Figure 5) to investigate the potential effects. Examining both sub- and supracritical gap lengths in mice (4 and 6 mm, respectively), aligned collagen was found to produce a notable improvement in the extent of PNI recovery over nonaligned collagen and saline-filled conduits. Over a gap length of 6 mm, magnetically aligned collagen and laminin hydrogels also resulted in improved functional recovery compared to nonaligned gels. However, it was noted that the extent of recovery, though an improvement over nonaligned gels and unfilled NGCs, was still relatively low. Regeneration was further impeded when hydrogels were more heavily crosslinked, further emphasizing the importance of a permissive environment for neurite growth.

A hydrogel comprised of amphiphilic nanofibers managed to achieve similar fibril orientation by using the fluid shear induced during the filling of a poly(lactic-co-glycolic) acid (PLGA) NGC. In vivo studies of a 12 mm defect in a rat showed that this hydrogel filler performed better than a hollow conduit and also experienced better sensory recovery compared to the autograft, although overall autograft performance was superior.

The general consensus of existing literature suggests that the presence of a hydrogel filler is beneficial for nerve defects larger than the critical gap length, although the regenerative capacity is still lower than the standard autologous graft. There are also indications that the density and structural organization of the hydrogel fibrils directly influence regenerative performance. Lower density environments and those with aligned fibrils for structural guidance showed improved neurite extension, suggesting reducing the impediment of invading tissue and introducing topographical cues are important design considerations for 3D environments created to enhance PNI recovery.

4. Fibrous Interluminal Fillers

Alternative methods for providing internal structure within the lumen of an NGC have also been investigated and focused on providing a more permissive environment for neurite and cellular infiltration. Taking inspiration from in vivo observations of the PNS ECM, many groups have introduced longitudinal guidance structures within the lumen in the

---

Figure 5. Magnetically aligned hydrogels. Fibrin hydrogels, made with fluorescently labeled fibrinogen, were imaged with confocal laser scanning microscopy. A) Isotropic fibrin gels were used for comparison. B) Longitudinal fibril formation in fibrin. C) As seen under bright field microscopy, neurite outgrowth from dorsal root ganglion (DRG) neurons was highly directional in magnetically aligned fibrin. Oriented neurite outgrowth from DRG neurons also occurred in D) aligned collagen gels, compared to E) isotropic collagen gels. In panels (D) and (E), neurites are stained green, SCs are stained red, and co-localized neurites and SCs appear yellow. Adapted with permission.
form of aligned fibers. These environments are typically more open and permissive compared to hydrogels; however, the approaches outlined in this section simply introduce fibrous structure as a filler. No additional control is exacted over fiber placement or the resulting macrostructure, with modulation of neurite growth induced by the presence, but not the explicit organization, of the residing fibers.

To facilitate this discussion, a distinction based on diameter is introduced: fibers with diameters of 10 µm or greater are referred to as filaments; fibers below this dimension are approaching the nanoscale and are therefore referred to as nanofibers. Because of the number of studies involving filaments as NGC fillers, these are further separated into filaments made from either synthetic material or natural ECM proteins.

### 4.1. Synthetic Filaments

One of the first reports of aligned fibers within an NGC was studied in 1946 by Weiss and Taylor. Using a variety of materials, parallel fibers of ~30 µm diameter were placed within a tube and held in place by coagulated blood (similar to fibrin gel). These were then sutured within a 20 mm defect in a cat animal model. Initial observations using glass fibers found that they tended to cluster, obstructing cell invasion and growth; furthermore, the mechanical mismatch and micromotion of the stiffer materials also led to failed regeneration. However, softer fibers of cellulose or Nylon resulted in successful regeneration and functional recovery, although it was found that in some cases fiber clustering was still experienced. This was accompanied by limited cell infiltration, indicating that regions of high fiber density still had the capacity to occlude regeneration. Some materials resulted in fibrotic tissue surrounded the fibers, within which no axons or SCs could be found. Nevertheless, regeneration was well supported and exhibited improved aligned neurite growth compared to a hollow tube filled with blood alone. This initial attempt highlights not only the promise of this technique, but also the limitations in terms of appropriate material selection and maintenance of fiber organization.

The advent of new biomaterials and advances in biofabrication prompted a revisit to this approach, initially with the introduction of relatively large polyimide filaments (250 µm in diameter) within the NGC lumen. This was originally motivated as a means of both directing cell infiltration and migration and for stabilizing endogenous fibrin matrix formation. Observations from the regeneration of a critical 10 mm defect in a rat found that fast resorbing fiber types improved the degree of regeneration, attributed to macrophage activation from the degradation of the filaments. The most promising among the materials used was catgut, which experienced both the maximal nerve regrowth and the most degradation and macrophage response. Furthermore, the presence of interluminal filaments for a supracritical gap defect (15 mm in rat) resulted in observable regeneration compared to none for a saline-filled conduit. Interestingly, this more challenging regenerative environment also abolished any observable differences between filaments types; simply the introduction of such an interluminal structure proved to be sufficient to promote regeneration.

Attempts to optimize filament packing density found that neurite growth was impeded when too many fibers were included. Packing densities ranging from 1.7% to 24% were examined (respective open space available ranging from 98.3% to 76% of the original NGC area). Not only did more filaments occupy more space, but also large areas were taken up by de novo connective tissue formed around the large fibers to further occlude neurite growth. Of note, in this study, catgut filaments displayed the poorest performance in this context, where a high filament packing density combined with the previously mentioned macrophage response resulted in the merging of newly formed connective tissue that was completely void of axons.

A later study employed smaller poly-1-lactide microfilaments (40–100 µm in diameter), again examining the role of packing density for different defect lengths. This study confirmed that the presence of filaments was beneficial for sub- and supracritical gap lengths, compared to a saline-filled NGC. It was also found that a lower packing density of 3.8% was best for filaments of this scale; filaments were again encapsulated by macrophages and neurite growth appeared to be occluded by subsequent growth of connective tissue. In particular, increasing the number of filaments results in an increase in nerve cable diameter but a reduction in the number of actual myelinated axons. This emphasizes that such an increase in the number of larger scale filaments intensifies the macrophage response and produces a larger amount of consolidated fibrotic tissue, presenting a barrier to neural regeneration.

However, this work also highlighted the importance of filament organization, with some observed cases of the lowest packing density allowing filaments to rearrange and cluster. This was the attributed cause of failed regeneration in a few isolated cases. In a follow-up study, Matrigel was used to stabilize filaments with a packing density of 7.5% within both silicone tubes and porous polyacetic acid (PLA) conduits. Although no autograft control was implemented in the study, the incorporation of PLA fibers produced successful recovery over an impressive 18 mm gap for both conduits, with the PLA guide experiencing improved quality of recovery; guides with only Matrigel achieved markedly lower performance.

### 4.2. ECM Filaments

Turning to natural ECM materials, Itoh et al. prepared collagen-based fibers of a similar diameter (100–150 µm) which were placed within an NGC and compared this to a collagen-gel-filled NGC. The collagen filaments were prepared by mechanically elongating and air-drying columns of collagen gel. At a packing density of ~5% (95% open area), NGCs with collagen fibers were found to have successful regeneration compared to both saline- and collagen-filled conduits. The axons and SCs were found in the intercollagen filament spaces, with fibroblasts/macrophage in and around the collagen fibers; larger fibers or swelling was noted to be a concern for obstructing growth. A decrease in collagen fiber size (70 µm fibers) with an increase in packing density to 12.2% also produced sufficient regeneration and functional recovery. This possibly suggests that the use of smaller filaments made of natural materials allows for a greater
packing density and more effective PNS regeneration. However, the use of a subcritical 10 mm defect size in both studies precludes any form of comparison with previous studies. Yet, the reported bridging of an 80 mm gap in a dog model using collagen filament interluminal filler suggests this approach is a worthwhile avenue of investigation.[31,173]

Even smaller collagen fibers derived from bovine skin were employed, this time without the protective encapsulation of a conduit.[174] About 2000 collagen fibers, each 20 µm in diameter, were assembled to form a free-standing fibrous graft with an approximate packing density of 10%. Placed in a 20 mm long PNS defect in a rat, this approach produced a similar degree of outgrowth compared to an autologous implant. However, many macrophages were present to degrade collagen fibers, and neurites within the autograft were comparatively larger and more mature. Furthermore, no functional recovery was noted for either implant, attributed to the insufficient time for complete recovery over this 8 week study. A follow-up study with a 30 mm defect over 12 weeks reported functional recovery, where the use of 4000 bundled fibers showed a slightly better muscle actuation than 2000 fibers. Although the lack of a conduit boundary makes an accurate estimate of packing density difficult, regeneration into the less dense scaffold (2000 fibers) resulted in fewer, more mature axons. In comparison, more axons with smaller diameters were observed in the denser scaffold (4000 fibers). Again, this suggests the importance of presenting an adequately and finely tuned permissive environment in order to elicit an optimal regenerative response.

4.3. Nanofibers

The structural fibril elements within the native ECM are known to range from ~40 to 100 nm,[163] while collagen fibrils that are newly formed in response to PNI can be as small as 25 nm in diameter.[26] This stark contrast to the filaments previously described underscores the technical barriers to creating equivalent biomimetic nanofibers. A reliable method to produce continuous fibers that can scale this and provide a degree of control over fiber orientation is electrospinning (ESP), capable of achieving diameters within the micrometer to sub-micrometer range.[175] This is an electrostatically driven method where nanofibers are formed from a charged liquid polymer solution or melt that is ejected through a capillary tube toward a grounded collecting target, quickly producing a large number of nanofibers in the form of a nonwoven mesh.[176] This versatile technique can form fibers out of a wide range of materials, including natural and synthetic polymers, composites, and ceramics.[175] Varying parameters such as solution viscosity and concentration, voltage, and feed rate, can control the nanofiber size, while adjusting the grounded collector can control fiber orientation.[176] For further details about how to control nanofibers morphology and alignment, as well as how to control scaffold architecture, one may refer to the works of Xie et al.[177] Chew et al.,[178] and Murugan and Ramakrishna.[179]

ESP fibers have been shown to provide effective nanotopographical cues for neurons, discussed thoroughly by Spivey et al.[180] A number of in vitro studies have assessed the response of neurons and supportive glial cells to fiber diameter, orientation, density, and possible functionalization.[176,181–190] A summary of relevant studies reveals that directed neurite outgrowth and SC migration can be induced with aligned fibers over a range of 1–5 µm in diameter. The permissiveness of the nanofibrous mesh, determined by the packing density of the fibers, was also identified as an important factor for promoting neurite extension and SC migration in vitro; while nanofibers ranging from 750 to 300 nm in diameter might better approximate native ECM fibrils, 1300 nm diameter fibers achieve a higher packing density and therefore present a substrate with more consistent instructive cues for improved cell response.[102,191]

Despite the extensive number of studies investigating neurite growth in vitro on ESP fibers, the application of electrospun fibers within the context of repairing peripheral nerve injury has mainly been restricted to the development and fabrication of the outer nerve guide conduits.[175,192] This may be attributed to the difficulty in the handling of delicate fiber constructs. We have recently shown that the fibers on the inner lumen wall of an electrospun conduit can begin to invade the inner lumen and further support regenerating neural tissue; however, the degree of luminal filling is minimal.[192] However, studies have explored a more direct implementation of aligned, electrospun fibers to more thoroughly fill the interluminal space. Koh et al. employed an electrospun filler of PLGA nanofiber yarn,[193] created in a manner similar to previous reports.[193] PLGA fibers from 200 to 500 nm were bundled together into yarn-like segments of 25 ± 5 µm in diameter, after which ~360 yarn segments were inserted into an NGC; this represents a packing density of ~10%. Although observations over a 12 week period in a 10 mm defect in a rat found higher density of axon growth in the autograft control, functional recovery of the lumen-filled guide was found to be superior.

The Bellamkonda laboratory has also applied ESP fibers as interluminal fillers, inserting multiple thin films of 400–600 nm poly(acrylonitrile-co-methacrylate) (PANMA) fibers, aligned or random, into an NGC. The initial study employed 10–12 sheets within an NGC, representing an approximate packing density of < 9%, and applied these constructs to a 17 mm nerve defect in rats.[188] The sheets of aligned fibers were found to effectively support neurite growth to a similar degree compared to the autologous control implant. However, fiber-filled conduit exhibited a segmented growth with respect to the cross section of the regenerated nerve cable compared to both the autograft and normal nerve, with distinct regions of neural cells juxtaposed to regions of non-neuronal cells; in contrast, the normal nerve and the autograft displayed evenly distributed neuronal cells. Growth through both implants resulted in neuromuscular junction formation and a return of function, although recovery was slightly higher in the autograft.

A follow-up study by Clements et al. investigated a similar platform, but reduced the number of ESP films.[194] Furthermore, fiber films were integrated into the conduit to hold them in place, allowing for a more precise strategy for film placement and exacting a degree of control over the macroscopic arrangement of fibers within the lumen. Two arrangements of ESP films were trialed: a single film inserted into the NGC, bisecting the cross-sectional area of the lumen into...
two semicircles (Figure 6A); and three films inserted to form a Z pattern across the face of the lumen, separating it into four distinct luminal regions (Figure 6B). These fibrous interluminal films occupied less than 0.6% of the NGC cross-sectional area. Results showed that the three-film conduit exhibited a larger cross-sectional area of the regeneration nerve cable, accounted for by the increased interluminal surface area provided. However, the single-film conduit experienced more axonal growth, with better self-organization of the nerve cable tissue and improved functional recovery. This suggests that the arrangement of additional electrospun sheets contributed to fragmented ingrowth of the regeneration nerve, with the thin films acting as a barrier to spontaneous tissue organization and resulting in reduced regenerative capacity. Unfortunately, drawing conclusions about the relative performance of these conduits is not possible due to a lack of autologous graft controls performed.

These findings highlight that a construct which presents a highly permissive environment for tissue infiltration and, as well, exhibits instructive topographical cues may still prove detrimental to nerve regeneration. This suggests that the design of an interlumen filler and its macroscopic architecture must either create an environment which does not inhibit tissue self-assembly or more closely mimics the endoneurium as with the autologous nerve graft.

5. Structurally Patterned Interlumens

The previous sections have outlined how simply providing biologically relevant bulk fillers as in the case of hydrogel fillers, or basic structural elements as with fiber fillers, act to improve regeneration when compared to a nonfilled NGC; this was particularly evident when gap defects over a critical size were treated. However, performance of these fillers failed to match that of the autologous nerve graft both in the return of function and in the quality of the regenerated tissue.

Many groups have pursued the creation of a more organized interlumen structure over the past 35 years, resulting in many attempts to design and fabricate a synthetic scaffold with optimized structural architecture capable of enhancing PNS regeneration. There is a recurring theme in literature that an interluminal structure hinders growth if the macroscopic architecture is not sufficiently permissive or does not match architecture of the native PNS ECM; as described previously, this is comprised of channel-like endoneurial structures ranging in diameter from ≈2 to 20 µm and delineated by longitudinally aligned collagen fibers, forming anisotropic pores that make up ≈50% of endoneurium cross-sectional area. Most fabrication approaches focus on creating continuous channels or interconnect anisotropic pores of varying dimensions, which provide passage of neurite growth from the proximal to the distal stump of a transected nerve.

While some techniques have been evaluated in vivo, others are still in stages of development. Outlined below are various methods cited as viable means of creating patterned and structurally relevant 3D scaffolds toward constructing nerve guidance conduits with an unprecedented degree of complexity. Broadly speaking these techniques have been classified according to (i) template molding, (ii) unidirectional freezing, (iii) sacrificial molding, (iv) self-assembly, and (v) direct writing.

5.1. Template Molding

A traditional approach to producing a polymer scaffold with designed structure is the molding of a polymer melt or polymer solution over a negative mold template, followed by mold extraction to produce a polymer cast. For the creation of an anisotropic luminal filler, the mold is typically in the form of an array of parallel cables or wires to subsequently create a scaffold with a controlled arrangement of parallel channels. An example of this approach was first reported by Hadlock et al. but has since been reported by many others (see Figure 7).

The size of the channels, typically ranging from 60 to 500 µm, is restricted by the mechanical stability of the molding wires used. The typical size of these structures precludes any parallels drawn between the native endoneurium, with these constructs often deemed multilumen conduits instead. These are conceived to provide more surface area for ingrowing tissue and to allow for ingrowth to be organized into fascicular-like subunits. An initial study used PLGA injected into a mold at lower pressure to form a multilumen conduit with five 500 µm luminal channel guides, which showed promising tissue growth over a 7 mm defect in a rat, although functional recovery was not assessed.

A thorough study by Yao et al. evaluated similar multilumen constructs made from collagen, implanted in a 10 mm defect in a rat. Comprised of either 2, 4, or 7 channels, with diameters of 530, 530, or 410 µm, respectively, it was found that the fascicle-like growth induced by the four channel multilumen graft exhibited larger axons and increased myelination compared to the commercially available single lumen graft; this was also reflected in slightly higher compound muscle action potentials, though the difference in improved functional recovery was not statistically significant. However, the autograft implant was found to have a consistently higher number of myelinated axons and markedly better degree of functional

Figure 6. A) A cross section of a nerve conduit with a single ESP film inserted, providing additional surface area and topographical guidance. B) Arrangements were also created where three films were inserted, forming a Z-like pattern to create four distinct interluminal regions. Reproduced with permission. Copyright 2009, Elsevier.
recovery. The relatively poor performance of the multilumen conduits results could stem from the only 20–30% open area they present, which poses a conspicuous barrier to neural tissue infiltration.

Recent reports combine the use of template cables to form orderly microchannels within an electrospun fibrous matrix, intended to create a highly biomimetic interluminal structure for PNS recovery. [202,203] Either a sheet of aligned electrospun fibers was directly deposited onto a planar array of parallel cables (either suture material or fishing line) and then rolled up or the cables were introduce at interval while the fibrous sheet was rolled up. Both approaches formed a cylindrical guide comprised of a fibrous mesh. Removing template cables created an architecture of through-channels framed by aligned nanoscale polymer fibers. This has produced microchannels ranging from 130 to 300 µm in diameter, similar in scale to previous molding attempts. However, the channels closely approximate the observed structure of the endoneurium, and channel density is greater than other molding methods, providing ≈50% available open area for nerve ingrowth. Although the mechanical instability of the resulting thin ESP fiber walls may prove detrimental, no in vivo studies have yet been performed with this construct to assess clinical performance. Regardless, these initial reports represent a novel and promising scaffold fabrication strategy with high potential for creating an effective biomimetic interluminal filler.

5.2. Unidirectional Freezing

The freezing of a hydrogel solution and subsequent water sublimation has been a long-standing and well-described method for creating a porous foam structure from a hydrogel solution.[204,205] If the rate of ice formation within a hydrogel is sufficiently slow, the solute material of the hydrogel will be pushed out of the way and excluded from the advancing ice crystal. Once freezing is complete, the hydrogel solute is confined to the boundary regions between opposing ice crystals. Upon sublimation, the solute solidifies in place to form a foam with a honeycomb-like structure. By controlling general parameters of the freezing process, pore diameters ranging from 95 to 150 µm are possible.[206]

This has been used to create isotropic collagen sponges and alginate sponges to be used as interluminal fillers for PNS repair.[31,207–210] Although the pores lack directionality, histological analysis of implanted scaffolds revealed that their scale, structure, and interconnected nature were able to induce successful innervation. Collagen sponges achieved higher action potential and conduction velocities compared to interluminal collagen fibers, although this difference was not statistically significant.[31] Histological analysis and functional evaluation also revealed that alginate sponges were able to repair a 50 mm defect in a cat,[210] even when simply placed within the gap and not retained within a nerve conduit.[209] However, comparison is hampered due to a lack of control groups in these studies.

Additional work has shown that the creation of pores with a bias direction and improved interconnectivity formation is possible via anisotropic ice crystal formation within a hydrogel, achieved by inducing a thermal gradient to create a unidirectional freezing process (Figure 8).[205,211–214] A study by Hu et al. provided a clear improvement in regeneration between collagen–chitosan grafts with anisotropic and isotropic pores, implanted in a 15 mm defect in a rat model.[215] The anisotropic pores created in this study were 34 µm on average, closely approximating both the structure and scale of the endoneurium. In general, regeneration through anisotropic collagen–chitosan grafts was significantly better compared to grafts with isotropic pores, with larger, more myelinated axons and action potentials with higher amplitudes and faster conduction velocities.[215] Furthermore, the anisotropic graft approached the performance of the control autologous graft.[215–217]

Similarly, positive results have been produced with pure collagen interlumen that has been anisotropically frozen in a similar manner.[218–220] The autograft control again had slightly larger axons and thicker myelination. Scaffolds prepared to degrade faster managed to approach these values to
Although the dimensions for these studies are still considered open area, throughout which successful growth was observed. Fibers using tetrahydrofuran. This created a scaffold with a 44% formed within agarose via sacrificial removal of polystyrene fibers using acetone. [221] This produced scaffolds with up to 40% of open area available for ingrowth. Though no PNS application has yet been trialed, this matrix has produced promising results in vivo for spinal cord repair studies.

5.3. Sacrificial Molding

An alternative method of molding polymer scaffolds relies on embedding a sacrificial molding material within a polymer matrix, forming an interlumen architecture by dissolving away the mold. Assuming a polymer solution is used for the casting process, this requires the use of a multisolvent system whereby the solvent for the scaffold material is a nonsolvent for the mold material and vice versa. Such a system was first reported by Flynn et al., who used to create 200–400 µm channels within poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogels by selectively leaching out embedded polycapro lactone (PCL) fibers with acetone. [221] This produced scaffolds with up to 40% open area available for growth. Despite such constructs supported cell growth in vitro, no follow-up in vivo study has been reported for this particular scaffold. [222]

Other solvent systems have also been employed, though primarily for the development of scaffolds for spinal cord repair. [223–226] A parallel array of 200 µm diameter channels were formed within agarose via sacrificial removal of polystyrene fibers using tetrahydrofuran. This created a scaffold with a 44% open area, throughout which successful growth was observed. Although the dimensions for these studies are still considerably larger than the 2–20 µm observed in the endoneurium, they provide working examples of a possible solvent/nonsolvent system suitable for this approach.

A recent report by Scott et al. describes the use of a similar nonsolvent/solvent system to create a likewise parallel array of channels ranging from 15 to 150 µm within a fibrin gel. [226] Using either poly(methyl methacrylate) (PMMA) or cellulose acetate fibers formed by melt spinning, these embedded fibers were later removed using acetone. This represents the creation of microchannel structures that most closely approximate the dimensions observed in native endoneurial tissue. Initial in vitro testing using dorsal root ganglion (DRG) explants revealed that all ranges of channel sizes promoted a promising degree of ingrowth, though in vivo performance remains to be validated.

An innovative solvent/nonsolvent system has relied on the use of sugars (also deemed carbohydrate glass) as the soluble template. [227–229] Various methods have been employed to embed the sacrificial cables to create the final polymer matrix. Initial approaches drew fibers from a caramelized sugar bath to produce sacrificial cables ranging in diameter from 8 to 200 µm, after which template fibers were encapsulated within the polymer matrix to avoid premature dissolution. [227,228] This has been achieved by dip-coating fibers into a nonsolvent polymer solution, such as poly-L-lactide (PLLA) dissolved in chloroform. [227] The sugar template from these coated fibers can be leached with distilled water to form standalone tubules or assembled into a parallel array of coated fibers and undergo leaching; these assembly of tubules present an estimated open area of ≈80%. The in vitro work performed thus far shows that these scaffolds promote neurite extension and guide SCs’ infiltration. The final arrays were also reported to be irregular in shape, and the mechanical stability of these constructs was also not explicitly stated.

Alternatively, fibers have also been coated with polymer microspheres, with these fibers later assembled into a parallel array. [228] Applying pressure to this assembly causes the microspheres to merge, creating a single polymer matrix with embedded fibers. After leaching in distilled water, scaffolds have been produced with up to 40% of open area available for ingrowth. Though no PNS application has yet been trialed, this matrix has produced promising results in vivo for spinal cord repair studies.

More recently, Lee et al. have produced an implanted nerve conduit that involves the electrospinning of oriented fibers onto a planar parallel array of 250 µm sucrose filaments. [230] After deposition, this composite sheet of nanofibers and filaments was rolled up to form a stable guide. Though the channels are much larger in scale than in the native endoneurium (see Figure 9A), implantation in a 10 mm defect of a rat nerve produced favorable results. Regeneration was observed to occur in all cases, though the autograft still excelled in most metrics; with exception, improved motor recovery was observed when electrospun fibers were functionalized with cell adhesive molecules.
While the number of in vivo studies is limited for sacrificial template strategies, advances in 3D-printing of sucrose-based soluble templates could facilitate rapid prototyping of appropriate sacrificial lattice templates. Although no neural applications have yet been reported and the reported 150 µm channel size is much larger than endoneurial structures, the ability to precisely design and create sacrificial templates in this manner presents many promising possibilities.

5.4. Self-Assembly

Relying on a completely different mode of fabrication, the development of a hydrogel system in which aligned, parallel microchannels are spontaneously self-assembled represents a highly novel method of creating an anisotropic tissue scaffold. This approach relies on the nonconvective diffusion of ions into an ion-crosslinkable polymer solution, with alginate being an often-used biocompatible candidate. This simple and unique approach produces microchannels in the range of 10–77 µm and an open area from 5% to 30%; channel dimensions can be tuned by controlling the ions used in the crosslinking process and eventual additives to the polymer solution (see Figure 9B). Until now, in vivo studies focused on spinal cord repair, although in vitro studies have shown ingrowth of sensory neuron and SC invasion. However, the current fabrication process is limited in terms of controlling microchannel distribution and density, producing scaffolds that would pose a considerable physical barrier to the regeneration of PNS tissue.

5.5. Direct Writing

The methods of scaffold fabrication described so far require the creation of a molding template or rely on the spontaneous, uncontrolled formation of microchannels. More direct approaches exist to create a 3D matrix that incorporates microstructural patterns, such as additive manufacturing (AM), multiphoton ablation, or stereolithography, though each is accompanied by possible limitation. Biofabrication using AM technologies generally involves the serial deposition of polymer or hydrogel material (with or without cells) to 3D structures (see a recent review by Maida et al. and Zhu et al.). This approach relies on the deposition of relatively large filaments (>75 µm) to create tissue scaffolds. While the scale of the resulting structures is perhaps more appropriate to realize NGCs, production can be fast and reliable while its flexibility lends itself to increasingly complex, anatomically relevant designs. Initial studies on AM-deposited intraluminal structures show that, despite structures much larger than endoneurial features, a printed construct is able to promote neural regeneration across a 10 mm defect in a rat. Refinements to the printing process have already produce a more intricate luminal filler design that has been successfully evaluated in vitro, suggesting great potential as this technology continues to develop.

Using a multiphoton beam, sufficient energy for material ablation is imparted only to the focal point of the incident laser, with microchannel dimensions limited by the laser wavelength and the point-spread function of the focused radiation energy. In addition, this approach is limited to optically transparent materials that are susceptible to ablation. In vitro experiments have clearly shown the utility and flexibility of two-photon ablation in creating microstructure within a 3D culture environment, coaxing neurite outgrowth to follow ablated microchannels as small as 5 µm. Though the large-scale production necessary for the creating of an in vivo implant remains an obstacle, new high-throughput techniques are in development to make this method of fabrication a promising avenue for providing detailed design and fabrication of a microchannel matrix.

Stereolithography is another method suitable for large-scale fabrication of NGCs with interluminal structures. This approach rapidly cures a photo-crosslinkable polymer solution, layer by layer, to form complex 3D shapes. A number of studies have already shown the utility of this technology for creating NGCs’ internal architecture. Multilumen channels reported were on the order of 300–500 µm in diameter, although the minimum dimension of fabricated structures is primarily determined by the resolution of the translation stages used (see Figure 9C). Although Evangelista et al. recently reported discouraging initial trials with a hydrogel-based multilumen design, outcomes could be improved through design changes or a different material choice. One possibility would be a multimaterial multiscale approach that combines electrospun fibers within crosslinked polymer. Already shown to support neurite growth in vitro, this would allow one to incorporate nanotopography within a 3D construct of an...
6. Discussion

In order for a technological solution to be implemented within a clinical setting, its performance must not only be effective but also surpass the currently accepted treatment methods. Owing to an intrinsic ability to recover, the PNS often does not require intervention. Even in many cases where small defects occur, the simplest approach is shown to be the most effective. This is evidenced by hollow NGCs shown to facilitate levels of recovery which match an autograft when applied to nerve defects below the critical gap length. The application of further tissue-engineered solutions for these cases can even have a detrimental impact on recovery, such as for luminal hydrogel fillers described above. The introduction of interluminal fillers for more extreme injuries with longer gap defects does show a benefit over the basic hollow NGC. However, the various metrics and analyses used to determine performance make it difficult to directly compare different strategies. To be able to resolve the true impact of these approaches, attempts have been made to mathematically extrapolate past experimental outcomes into a universal model along with recent calls to establish standardized methods of evaluation for future studies. Regardless, the general consensus of literature addressed in this review indicates that homogeneous fillers are inadequate for enhancing PNS regeneration and require the incorporation of biomimetic microarchitecture in order to approach the recovery level achieved by the autograft.

Surpassing the performance of autografts will likely require NGCs to combine the implementation of biomimetic luminal fillers with other design elements and strategies not covered in this current review. One cannot overlook the importance of the housing NGC conduit, an essential component to most scaffold strategies. The outer NGC provides a mechanically stable platform to realize an enhanced neuroregenerative scaffold and facilitate the final surgical application. Although relatively basic compared to some of the scaffolds described, not all NGCs are equal. Tuning the properties of the NGC can have a significant effect on its regenerative capacity, including aspects such as mechanical stiffness, porosity, and biodegradability. For a concise overview of pertinent NGC design considerations, the reader is directed to the work of Jiang et al.

While the focus of this review is primarily on fabrication and the role of structural cues in NGC design, the incorporation of support cells (stem cells and SCs), cell adhesion peptides, and the delivery of diffusible biomolecules are additional strategies known to greatly enhance neurite growth. During PNS regeneration, distal SCs promote membrane-bound cell adhesion molecules as well as generate gradients of growth factors, both of which attract and guide neurite extension. This can either be recreated by seeding SCs or SC-like cells within the conduit or by emulating their presence, either through the conjugation of proteins onto a scaffold surface or by the implementation of diffusible cues through any number of established strategies. Bioconjugation strategies would also permit the incorporation of cell adhesion motifs to mimic natural ECM proteins, presenting another avenue by which neurite growth can be stimulated and controlled.

The combination of all of these elements culminates toward an increasingly complex guidance scaffold. Extrapolating trends outlined here, it can be expected that PNI recovery will continue to improve as scaffolds approach the level of structural and biochemical heterogeneity observed in native tissue. As scaffolds become more competent, developments and discoveries may find applications in other fields, such as providing treatment options for neuropathologies, enabling SCI repair, or facilitating neural interface development to improve neuroprosthetic control. Optimization of constructs as they become more multifaceted will require precise and systematic control over composition and structural organization. The approaches highlighted here provide an overview of current technological solution and provide a context for future biofabrication development.

Acknowledgements

The authors would like to acknowledge the funding support of the Natural Sciences and Engineering Council of Canada (NSERC) and the Province of Limburg.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

biofabrication, biomimicry, nerve guides, peripheral nerve regeneration, structural cues

Received: October 1, 2017
Revised: November 13, 2017
Published online: January 19, 2018

[1] C. Ide, Neurosci. Res. 1996, 25, 101.
[2] A. Hallgren, A. Björkman, A. Chemnitz, L. B. Dahlin, BMC Surg. 2013, 13, 39.
[3] F. F. A. IJpma, J.-P. A. Nicolai, M. F. Meek, Ann. Plast. Surg. 2006, 57, 391.
[4] G. Orive, E. Anitua, J. L. Pedraz, D. F. Emerich, Nat. Rev. Neurosci. 2009, 10, 682.
[5] J. T. S. Pettikiriarachchi, C. L. Parish, M. S. Shoichet, J. S. Forsythe, D. R. Nisbet, Aust. J. Chem. 2010, 63, 1143.
[6] M. M. Zaleska, M. L. T. Mercado, J. Chavez, G. Z. Feuerstein, M. N. Pangalos, A. Wood, Neuropharmacology 2009, 56, 329.
[7] S. T. Carmichael, Neurobiol. Dis. 2010, 37, 237.
[8] R. Richardson, A. Singh, D. Sun, H. Fillmore, D. Dietrich 3rd, M. Bullock, J. Neurosurg. 2010, 112, 1125.
[9] B. M. Aertker, S. Bedi, C. S. Cox, Jr., Exp. Neurol. 2016, 275, 411.
[10] A. E. Haggerty, M. Oudega, Neuromod. 2013, 29, 445.
[11] K. S. Straley, C. W. P. Foo, S. C. Heilshorn, J. Neurotrauma 2010, 27, 1.
X. Wen, P. a. Tresco, H. B. Wang, M. E. Mullins, J. M. Cregg, A. Hurtado, M. Oudega, M. H. W. Ooi, S. Hafeez, C. A. van Blitterswijk, L. Moroni, Mat. Horizons 2017, 4, 1020.

D. Ceballos, X. Navarro, N. Dubey, G. Wendelschafer-Crabb, W. R. Kennedy, R. T. Tranquillo, Exp. Neurol. 1999, 158, 290.

E. Verdú, R. O. Labrador, F. J. Rodríguez, D. Ceballos, J. Forés, X. Navarro, Restor. Neurol. Neurosci. 2002, 20, 169.

A. Li, A. Hokugo, A. Yalom, E. J. Berns, N. Stephanopoulos, A. Li, A. Hokugo, A. Yalom, E. J. Berns, N. Stephanopoulos, Tissue Eng., Part A 2006, 5789.

H. Wang, B. E. Layton, A. M. Sastry, Diabetes Metab. Res. Rev. 2003, 19, 288.

P. Weiss, A. C. Taylor, J. Neurol. Sci. 1946, 3, 375.

G. Lundborg, L. Dahlin, D. Dohi, M. Kanje, N. Terada, J. Hand Surg. Br. 1997, 22, 299.

N. Terada, L. M. Bjursten, M. Papoloizos, G. Lundborg, Restor. Neurol. Neurosci. 1997, 11, 65.

N. Terada, L. M. Bjursten, G. Lundborg, J. Mater. Sci.: Mater. Med. 1997, 8, 391.

T. Arai, G. Lundborg, L. B. Dahlin, Scand. J. Plast. Reconstr. Surg. Hand Surg. 2000, 34, 101.

L. B. Dahlin, G. Lundborg, J. Mater. Sci.: Mater. Med. 1999, 10, 549.

T. B. Ngo, P. J. Waggoner, A. A. Romero, K. D. Nelson, R. Murugan, S. Ramakrishna, S. Itoh, K. Takakuda, H. Samejima, T. Ohta, K. Shinomiya, S. Meiners, I. Ahmed, A. S. Ponery, N. Amor, S. L. Harris, V. Ayres, J. Xie, M. R. MacEwan, A. G. Schwartz, Y. Xia, M. T. Trombley, R. J. Gilbert, J. Neurosci. Res. 2005, 75, 2367.

Y. Fan, Q. Chen, R. Delgado-rivera, A. N. Babu, J. Biomed. Mater. Res., Part A 2006, 26, 605.

J. H. Wang, M. E. Mullins, J. M. Cregg, A. Hurtado, M. Oudega, M. T. Trombley, R. J. Gilbert, J. Neurosurg. 2009, 6, 16001.
[222] T. T. Yu, M. S. Shoichet, *Biomaterials* 2005, 26, 1507.
[223] S. Stokols, J. Sakamoto, C. Breckon, T. Holt, J. Weiss, M. H. Tuszyński, *Tissue Eng.* 2006, 12, 2777.
[224] T. Gros, J. S. Sakamoto, A. Blesch, L. a Havton, M. H. Tuszyński, *Biomaterials* 2010, 31, 6719.
[225] M. Gao, P. Lu, B. Bednark, D. Lynam, J. M. Conner, J. Sakamoto, M. H. Tuszyński, *Biomaterials* 2013, 34, 1529.
[226] J. B. Scott, M. Afshari, R. Kotek, J. M. Saul, *Biomaterials* 2011, 32, 4830.
[227] J. Li, T. A. Rickett, R. Shi, *Langmuir* 2009, 25, 1813.
[228] A. M. Thomas, M. B. Kubilius, S. J. Holland, S. K. Seidlits, R. M. Boehler, A. J. Anderson, B. J. Cummings, L. D. Shea, *Biomaterials* 2013, 34, 2213.
[229] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, M. H. Tuszynski, *Biomaterials* 2013, 34, 1529.
[230] J. B. Scott, M. Afshari, R. Kotek, J. M. Saul, *Biomaterials* 2011, 32, 4830.
[231] J. Li, T. A. Rickett, R. Shi, *Langmuir* 2009, 25, 1813.
[232] A. M. Thomas, M. B. Kubilius, S. J. Holland, S. K. Seidlits, R. M. Boehler, A. J. Anderson, B. J. Cummings, L. D. Shea, *Biomaterials* 2013, 34, 2213.
[233] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, M. H. Tuszynski, *Biomaterials* 2013, 34, 1529.
[234] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, M. H. Tuszynski, *Biomaterials* 2013, 34, 1529.
[235] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, M. H. Tuszynski, *Biomaterials* 2013, 34, 1529.
[236] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, M. H. Tuszynski, *Biomaterials* 2013, 34, 1529.
[237] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, M. H. Tuszynski, *Biomaterials* 2013, 34, 1529.
[238] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, M. H. Tuszynski, *Biomaterials* 2013, 34, 1529.
[239] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, M. H. Tuszynski, *Biomaterials* 2013, 34, 1529.
[240] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, M. H. Tuszynski, *Biomaterials* 2013, 34, 1529.