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Engineering Peripheral Nerve Repair

Laura Marquardt[1] and **Shelly E. Sakiyama-Elbert**[1,2,*]

[1]Department of Biomedical Engineering, Washington University, St. Louis, MO 63130, USA

[2]Division of Plastic and Reconstructive Surgery, Department of Surgery, Washington University School of Medicine, St. Louis, MO 63110, USA

Abstract

Current approaches for treating peripheral nerve injury have resulted in promising, yet insufficient functional recovery compared to the clinical standard of care, autologous nerve grafts. In order to design a construct that can match the regenerative potential of the autograft, all facets of nerve tissue must be incorporated in a combinatorial therapy. Engineered biomaterial scaffolds in the future will have to promote enhanced regeneration and appropriate reinnervation by targeting the highly sensitive response of regenerating nerves to their surrounding microenvironment.

Introduction

Currently there is a great disparity in functional outcomes between engineered biomaterials for nerve repair and the clinical standard of care, nerve autografts [1]. This disparity has led to a multitude of approaches to target the complexity of nerve regeneration. Biomaterials are currently being tailored to address these issues because currently marketed nerve guidance conduits (NGCs) cannot match the performance of autografts in large nerve defects (greater than 10 mm in rats, or greater than 30 mm in humans) [2]. Yet, an engineered construct capable of promoting neuronal survival, as well as axon extension and guidance is needed to provide equivalent functional outcomes to an autograft. This “off the shelf” alternative is desirable to prevent harvesting tissue that results in donor site morbidity and to improve upon the limitations of autograft recovery, where less than 25% of patients regain proper motor function and less than 3% regain sensation [3]. Current approaches focus on the sensitivity of regenerating axons to the surrounding environment, which includes surface topography, biochemical cues, and electrical activity. Surface topography has been well established as a mediator of axonal guidance and extension [4]. Thus, many groups have focused on incorporating architecture that mimics the native nerve into engineered constructs to better orient regenerating nerves and promote appropriate reinnervation. Neuronal survival and axon extension has been improved by functionalizing biomaterial scaffolds with neurotrophic factors (NFs) and extracellular matrix (ECM) proteins (or peptides derived from these proteins). Research in this field has shown the inclusion of factors found within native nerve tissue, using either natural or synthetic biomaterial scaffolds, yielded enhanced regenerative capacity. The inherent electrical activity of nerve

*To whom correspondence should be addressed: Shelly Sakiyama-Elbert, PhD, Department of Biomedical Engineering, Washington University, Campus Box 1097, One Brookings Drive, St. Louis, MO 63130, Telephone: (314) 935-7556, Fax: (314) 935-7448, sakiyama@wustl.edu.

has prompted the development of electrically conductive biomaterials that may promote increased axonal regeneration with electrical stimulation. Alone, each of these strategies may improve regeneration across moderate gaps, yet many studies described in this review have focused on combination therapies that incorporate of two or more of these elements (Figure 1). This review details current research in this field, in which the development of multi-faceted biomaterial scaffolds may improve the functional outcomes to the level of autografts or improve beyond autograft levels.

Engineering physical and topographical cues for neural guidance

Native nerve architecture includes an elongated, fascicular morphology that enables axonal guidance following injury through the formation of the Bands of Büngner. Bands of Büngner are formed by proliferating Schwann cells that help guide regenerating axons to target organs. Commercially available NGCs are often hollow tubes or nerve wraps that lack this native architecture, thus many groups have focused on developing materials that provide guidance within conduits connecting the proximal and distal nerve stumps after injury. Ribeiro-Resende *et al.* attempted to promote the generation of artificial Bands of Büngner through aligned collagen and poly-ε-caprolactone (PCL) filament constructs. Seeded with Schwann cells, these aligned microfilaments were capable of promoting enhanced, oriented outgrowth of dorsal root ganglia (DRG) neurites *in vitro* [5]. This study also found through combination of topographical cues, as well as what they termed “polarizing” differentiation factors, nerve growth factor (NGF), neuregulin-1, and transforming growth factor-β (TGF-β), they achieved increased Schwann cell orientation, which in turn provided better axonal guidance. The Hoffman-Kim group has focused on mimicking the native Bands of Büngner architecture through the development of Schwann cell imprinted molds [6]. Cell topographical molds were created from aligned Schwann cell substrates that were also capable of promoting highly aligned neurite outgrowth from DRG neurons *in vitro*. This group further developed conduits based off of this Schwann cell-mimicking topography that influenced DRG neurite extension, as well as cell migration patterns, which may prove useful *in vivo* [7].

Many groups have developed highly aligned, porous biomaterial scaffolds of natural [8–11] and synthetic materials [12–14] that aim to provide longitudinally, aligned substrates to guide regenerating axons. In addition to intraluminal porosity and topography, the effect of conduit porosity is important as increased porosity may decrease axonal regeneration toward the distal nerve segment. Oh *et al.* observed that conduits with nanopores increased longitudinal regeneration, whereas microporous conduits caused regeneration into the pores [15]. *In vivo*, Daly *et al.* showed aligned conduits aided regeneration of axons through the use of ultra-structured, grooved collagen fibers. Intraluminal collagen fibers with laser-fabricated, microgrooves reduced axonal mismatch with the distal nerve stumps compared to unstructured collagen fibers or hollow collagen conduits; however, functional recovery has yet to be tested [16].

One of the most popular methods of creating aligned biomaterial substrates is through electrospinning. *In vitro*, electrospun scaffolds have been shown to promote cell migration and guide neurite extension from DRGs [17]. Electrospun scaffolds are commonly fabricated from synthetic materials, such as PCL [18–20], poly-acrylonitrile (PAN) [21], and poly-L-lactic acid (PLLA) [22], and natural materials, such as silk, collagen, and blends of silk and PLLA [23,24]. *In vivo*, aligned electrospun fibers promoted significantly enhanced axon regeneration in a sciatic nerve injury model, as assessed by increased nerve fiber number, electrical activity, and motor reinnervation compared to randomly aligned electrospun fiber mats [21,24–26]. These studies show the importance in designing scaffolds

that provide structure similar to that of native nerve architecture, as well as topological guidance for regenerating axons to the distal target of innervation.

Enhancing biomaterial scaffolds with axon promoting factors

In addition to topographical cues, many engineered conduits now incorporate important growth factors and adhesion cues, such as NFs and ECM proteins. Laminin mediates cell survival, axon extension and cell adhesion through specific peptide sequences, IKVAV and YIGSR, as well as important integrin signaling so its role in nerve regeneration has been well studied [25,27–30]. Cao *et al.* developed linear ordered collagen scaffolds that have been modified with laminin by covalent attachment to promote axonal regeneration. In addition, laminin was used as a means for delivery of ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF) via laminin-binding domains (LBD). Laminin alone improved myelinated axon number *in vivo*, yet the controlled delivery of CNTF through the LBD, showed an additional improvement in axon regeneration and conduction velocity of the regenerating sciatic nerve [31]. Controlled delivery of BDNF and CNTF also showed improved compound muscle action potential (CMAP) activity of rat facial nerves [32]. The incorporation of biochemical factors, such as laminin, CNTF, and BDNF, indicate that while structural cues from the collagen scaffold are important, additional cues can further enhance functional outcomes.

ECM proteins that are native to nerve architecture have proven useful in enhancing neurite outgrowth *in vitro* and *in vivo*. Fibronectin is an ECM protein that is important for cell migration and adhesion via integrin binding to the RGD domain. Fibronectin has shown to promote neurite extension *in vitro* in combination with various polymer scaffolds, including aligned electrospun PAN-methacrylate, polyethylene glycol, and collagen [17,33]. Engineered elastin-like protein hydrogels, which contain RGD binding sites and mimic native nerve mechanical properties in a controlled manner, significantly increased neurite extension from DRGs *in vitro* [34]. These tunable hydrogels may prove useful in fabricating tailored biomaterial scaffolds that provide optimal adhesion properties for regenerating axons.

One of the best commercially available options for treating nerve defects, specifically long nerve gaps, are acellular nerve allografts. These are nerve grafts that undergo a decellularization process, either through chemical or thermal processing, that removes immunogenic, cellular components of the tissue [35]. This processing maintains most of the native nerve architecture composed of important ECM proteins, such as laminin and collagen, which can promote enhanced regeneration and functional recovery in combination with the structural cues in long term studies [36]. Acellular nerve allografts are also being used as a platform for delivery of cells and NFs. For example, Wang *et al.* has optimized acellular grafts to deliver bone mesenchymal stromal cells (BMSCs) to stimulate enhanced axon regeneration, and chondroitinase ABC to remove inhibitory molecules. This combination therapy stimulated secretion of NFs, such as NGF and BDNF, increased Schwann cell markers and angiogenesis markers, vascular endothelial growth factor (VEGF) and CD34, expression and decreased inhibitory chondroitin sulfate proteoglycans in the regenerating nerve. This approach increased myelinated nerve fiber number, myelin thickness, and axon diameter; again suggesting that while the acellular grafts are an excellent platform, combinatorial strategies can further enhance axonal regeneration and functional recovery through acellular grafts [37].

Many scaffolds, from both synthetic and natural polymers, have been functionalized to deliver NFs and ECM proteins through various chemical crosslinking methods. In Shepard, *et al.*, PEG hydrogels were functionalized to locally deliver viral vector constructs for NGF

via affinity peptides, which promoted increased neurite outgrowth from DRGs *in vitro.* These gels also encapsulated protease-secreting HT-1080 cells in order mimic infiltrating cells after injury that may degrade the hydrogel crosslinks and increase viral vector release [38]. Affinity peptides have also proven useful for the controlled delivery of NFs, such as NGF and GDNF, from fibrin matrices, which promoted enhanced motor regeneration, target reinnervation and functional recovery [39–41]. Both affinity-based peptides, as above, and chemical conjugation methods have been used to delivery NFs and ECM proteins in a controlled manner that may prove ideal for *in vivo* regeneration [42–46].

Stimulating nerve regeneration through conductive biomaterials and electrical stimulation

As previously described, many strategies are focused on mimicking native nerve attributes including architecture, protein composition, and NF delivery. Another strategy has focused on the inherent electrical excitability of neurons. *In vitro* and *in vivo*, electrical stimulation has shown to increase neurite extension and axonal regeneration [47,48]. Thus, engineering a biomaterial scaffold that is electrically conductive may improve regeneration and functional recovery following injury.

In vitro investigation has shown that using electrically conductive materials, such as polypyrrole (PPy) and polyaniline (PANi) in small amounts, combined with other well-characterized, degradable polymers, are capable of promoting enhanced neurite extension with low electrical stimulation. Song *et al.* demonstrated increased neurite extension area in complex geometries when photoresist patterns were doped with electrically conductive polymers, (PPy) as well as chemically conjugated NGF and poly-L-lysine/laminin [49,50]. The Schmidt group developed scaffolds in which NGF was chemically conjugated to PPy and PPy-PLGA scaffolds where it was found to increase the percentage of neurite expressing cells and the average PC12 cell neurite length *in vitro* with electrical stimulation [51–54]. PLLA-PANi scaffolds have shown promise for directing neural stem cell (NSC) differentiation, as electrical stimulation of PLLA-PANi scaffolds promoted elongated, neurite morphology of NSCs compared to unstimulated controls [55].

Polycaprolactone fumarate (PCLF)-PPy scaffolds were developed to promote increased neurite extension, where it was observed that only scaffolds formed via specific anions needed for PPy stabilization, naphthalene-2-sulfonic acid sodium salt (NSA) and dodecylbenzenesulfonic acid sodium salt (DBSA), can support cell adhesion, survival, and neurite extension [56]. These scaffolds were then fabricated into NGCs without any disruption of material properties. These scaffolds promoted enhanced neurite length and percent neurite-expressing PC12 cells with electrical stimulation and were capable of promoting aligned neurite extension in the direction of the applied electrical current [57]. *In vivo*, Huang, *et al.* demonstrated porous, biodegradable PPy-chitosan conduits increased regeneration and functional recovery following intermittent electrical stimulation. Conductive scaffolds in combination with electrical stimulation increased nerve fiber and myelinated fiber number, enhanced motor and sensory regeneration, functional recovery, and decreased muscle atrophy [58]. These electrically conductive scaffolds have shown promise in increasing nerve regeneration in large gaps with electrical stimulation; thus incorporating electrical conductive materials into chemically and structurally designed constructs may be necessary to promote enhanced functional motor and sensory recovery.

Remaining Challenges

While the current research shows great potential, regeneration across gaps greater than 30 mm remains a major challenge clinically particularly for patients who suffer multiple

injuries due to trauma. In addition to providing structural and biochemical cues, future combination therapies should focus on how these cues can be modulated spatially and temporally in response to the speed of regeneration. For example, the growth factor concentration at a given location in the distal nerve may need to increase early during the regeneration process to promote axon growth toward that site, and then decrease after the growth cones have passed to prevent axon trapping at that particular location thus allowing innervation of the target muscle. New methods that allow modulation of cues will be key to improving long-range regeneration and function.

Conclusion

Current strategies, focused on mimicking nerve structure and function, have shown vast improvement over unstructured, commercially available, hollow NGCs. However, to provide a microenvironment similar to a nerve autograft, engineered constructs must incorporate cues from native nerves including surface topography, biochemical signals, and electrically active tissue. Thus, any future development of NGCs of synthetic or natural materials will have to be a combinatorial approach that includes several of these aspects to target functional outcomes that match the clinical standard, autografts.

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Highlights

- Commercial nerve guidance conduits are insufficient to promote enhanced regeneration.
- Multi-faceted approaches are needed to mimic native nerve architecture and function.
- Engineered constructs with topographical cues facilitate aligned axonal regeneration.
- Delivery of native nerve biochemical and electrical cues improve functional outcomes.

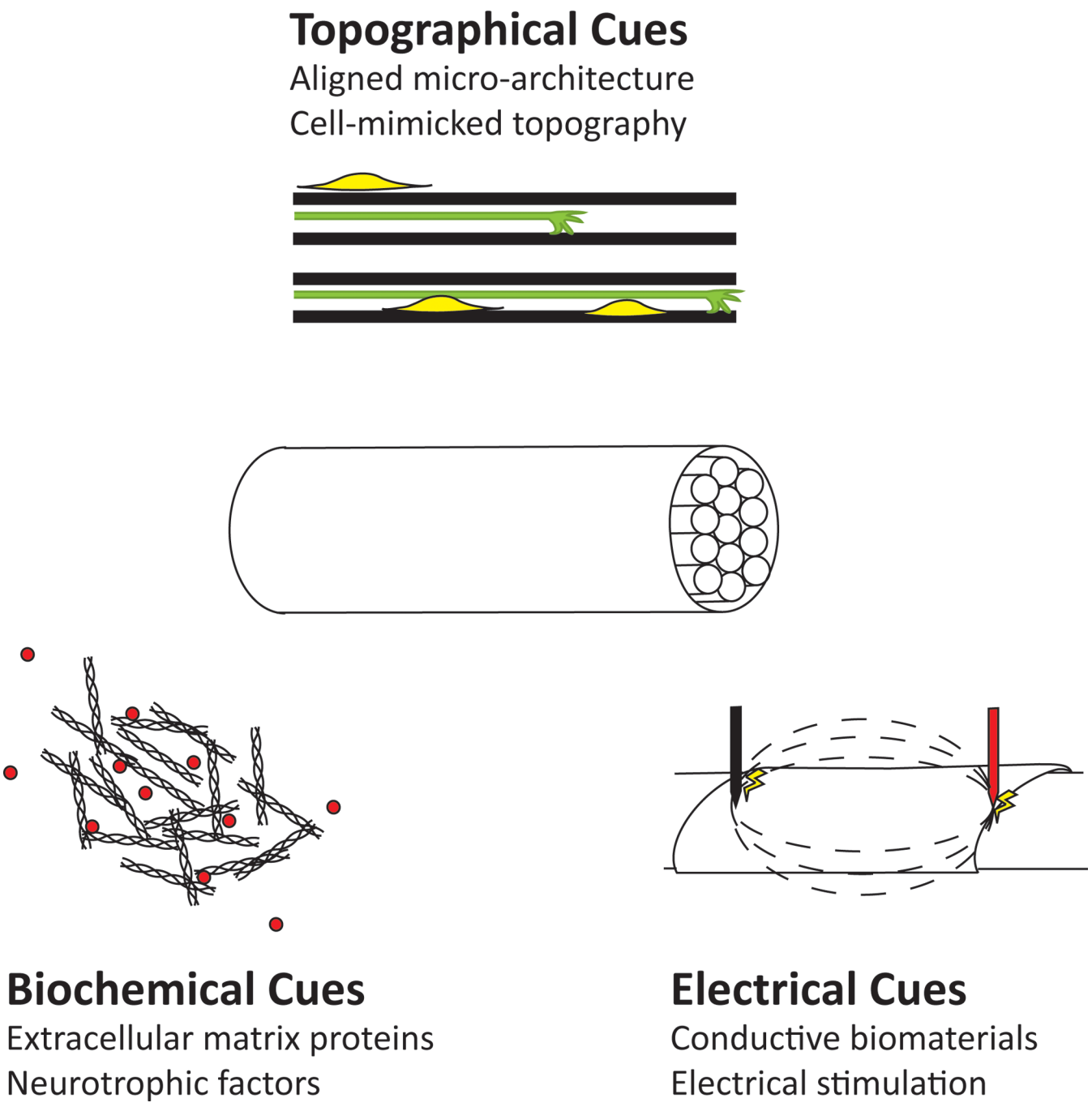

Figure 1.

In order to engineer a nerve guidance conduit that promotes enhanced functional recovery, many aspects of native nerve architecture and function must be incorporated in the design. Regenerating axons are sensitive to the microenvironment of nerves that includes topographical cues, growth promoting biochemical cues such as ECM proteins and neurotrophic factors, and the excitability of neurons through electrical stimulation.