DESIGNING AN IDEAL 3D-BIOPRINT CONDUIT FOR AXONAL REPAIR AND REGENERATION: A NEUROSURGICAL PERSPECTIVE

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*Peripheral nerve injuries occur through three mechanisms, specifically, crush, compression or transection. Disruption of communication between the peripheral and central nervous system follows and leads to motor and sensory deficits. Peripheral nerves in humans have a limited capacity to self-regenerate following injury, which makes nerve transfer the current gold-standard for treatment. Functional nerve regeneration is contingent on several factors ranging from span of injury and the age of the patient. Bioprinted nerve guidance conduits are an emerging candidate for treating peripheral nerve injuries. To optimize the performance of nerve guidance conduits, a firm understanding of neurobiology and the pathophysiology following injury is necessary. This article provides an overview of nerve regeneration and the desirable features when designing a nerve conduit from a neurosurgical perspective. **Biomed Rev 2019; 30:1-13***

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INTRODUCTION

The peripheral nerve injury remains one of the most common injuries encountered in the trauma and clinical setting. It is estimated that 20 million Americans suffer from peripheral nerve injury due to chronic diseases such as multiple sclerosis or more commonly, diabetes mellitus, as well as trauma (4). Majority of peripheral nerve damage being at the upper limbs in the order of frequency: radial, ulna and median. Whereas in the lower limb, comes in the order of frequency: sciatic, peroneal and tibial/femoral (5). Approximately 150 billion USD are spent yearly going towards the care and treatment for peripheral nerve damage (3). Besides the financial burden, the majority of the population who were affected by peripheral nerve cases were ages between 18 to 35 years old (6). Typical acute symptoms of peripheral nerve damage include sensory or motor loss of limbs, while chronic symptoms include neuropathic pain and psychiatric issues (3). As a consequence, peripheral nerve damage can significantly reduce the quality of life in many young and/or previously functionally normal patients.

Abbreviations

ADSC, Adipose-derived stem cells
 BDNF, Brain-derived neurotrophic factor
 FFMT, Free functioning muscle transfer
 FGF, Fibroblast growth factor
 GDNF, Glial cell-derived neurotrophic factor
 GFRA-1, GDNF family receptor alpha-1
 GFRA-2, GDNF family receptor alpha-2
 GGF, Glial growth factor
 IL-1 β , Interleukin-1 beta
 NGF, Nerve growth factor
 NT-3, Neurotrophin-3
 NCAM, Neural cell adhesion molecule
 PMP22, Peripheral myelin protein 22
 RAGs, Regeneration-associated genes
 SC, Schwann cells

The primary goal of nerve regeneration is to regain baseline sensory and/or motor function to the target organ. However, such regeneration greatly depends on the nerve gap, neuroma and scar tissue formation (3). Given that small nerves grow at a rate of 1 mm/day, while large nerve grow at a rate of 5 mm/day, the accepted time window for peripheral nerve regeneration is 12-18 months with some patient taking as long as 26 months (3). While this regeneration is happening, atrophy of the denervated muscle fibers develops immediately and after

4 months, 60-80% of the muscle mass has been lost (sarcopenia). This leads to decrease in end organ function even after nerve regeneration (7). However, this concept is subjected to debate. As Sulaiman *et al.* have demonstrated there are, in fact, a reduced number of motoneurons that regenerate their axon to the muscle fiber unit, and not the muscle unable to accept the innervations (8). Also, that previous studies have failed to account for growth of daughter axons from a single nerve fiber, and that previous research did not directly estimate the number of injured neurons that regenerated into the distal nerve stump, those that reinnervate the muscle fiber (8). Despite the need to reduce peripheral nerve regeneration time, currently there are no treatments to achieve that. Therefore, most research has been focusing on reducing this time frame.

The gold standard currently for peripheral nerve repair is direct surgical nerve repair with epineural microsutures (3). Other surgical techniques include grouped fascicular repair, free functioning muscle transfer (FFMT) and interpositional autologous nerve grafts (9). In an autologous nerve graft, the idea is that it will undergo Wallerian degeneration which serves a mechanical guidance and create a supportive structure for ingrowing axons (3). However, surgical nerve repairs have proved problematic, given that a nerve must be sacrificed, and it is predisposed to complications such as neuroma and fibrosis (3). Nerve transfer, where a proximal peripheral healthy nerve is sutured onto the damaged nerve has shown clear benefits with decreased regeneration time (7). However, finding an expandable nerve near the target tissue with large enough fibers may be a challenge to a lot of patients (10). Finally, FFMT directly transfer a healthy muscle and its neuromuscular bundle to a new location for a new function (9). This procedure is complex and very invasive that is only as the last option for reconstruction.

Current translational research focus much on enhancing axonal regeneration (Fig. 1) through growth factor action and 3D printed nerve conduit. Growth factors such as neurotrophin-3 (NT-3), nerve growth factor (NGF), fibroblast growth factor (FGF), glial cell-derived neurotrophic factor (GDNF), glial growth factor (GGF), ciliary neurotrophic factor and leupeptin have all been implicated in peripheral nerve regeneration (11). Nerve growth factor is important in nerve survival and outgrowth of neurites (12). In fact, NGF seeded conduits have better functional outcome than autograft in rabbit facial nerves (12). There are FDA approved commercially available nerve conduits on the market, but they have been shown not being effective in extensive lesions or larger nerves gaps greater than 3cm (13). 3D printed nerve conduit allow design and

create custom 3D scaffolds for nerve regeneration. Using “bioink” which include both synthetic and neutral material such as polyaniline (PANI), allow the grafting of certain cells such as Schwann cells (SC) and neurotropic factors into the conduit itself to encourage regeneration. Furthermore, scaffold or conduit fabrication incorporates nanostructural and microstructural design components for regenerating complex tissues with patient-specificity (14).

Figure 1. Peripheral Nerve Anatomy. Reproduced from (3).

NERVE INJURY CLASSIFICATION

In 1942, Seddon made the earliest classification of peripheral nerve injury, in which he distinguished three different types of nerve injury: Neurapraxia, Axonotmesis, Neurotmesis (15). Neuropraxia is the least serious and describes conduction blocks which leads to temporary sensory or motor loss. This type of injury is most commonly found in athletes. Axonotmesis is the next level of damage where the axon is irreversibly locally damaged, most commonly due to crush, nerve stretch and percussion injury. Finally, neurotmesis means the myelin sheath, axon and surrounding stroma are all injured, and no regeneration will occur unless there is surgical intervention. Such damage is most commonly associated with serious sharp or traction injuries (16).

In 1951, Sunderland (Fig. 2) expanded the peripheral nerve injury classification to five grades which provide better overall prognosis predictions (Table 1). Sunderland's grade I and II can recover completely, while grade III recovers partially, and grade IV and V require surgical intervention (3). Compared to the Seddon's classification, grade I corresponds with neuropraxia, II to axonotmesis and III, IV and V to neurotmesis

(17). Finally, in 1988, Mackinnon and Dellon described a new type of peripheral nerve injury named type VI, which is a combination of Type II to IV. This type of injury may be the most common in penetrating trauma and fracture, thus more reflective of real-life peripheral nerve damage (18). Currently, Sunderland's classification can only be diagnosed histologically (19).

Figure 2. Sunderland classification of peripheral nerve injury. Image courtesy Cleveland Clinic, 2006. Illustrator, David Schumick, BS, CMI (1).

PATHOPHYSIOLOGY INJURY AND REGENERATION

Peripheral nerves can be injured in many ways including inflammation, demyelination and trauma. In the internal neuronal network, there is constant anterograde and retrograde transport and communication. Once these communications have been interrupted or that the neuronal lipid layer is damaged, unless rapidly repaired there will be rapid and irreversible programmed cell death. Axonal degeneration happens both at

Table 1. Comparison of Sunderland and Seddon peripheral nerve injury classifications

Sunderland (20)	Seddon (21)	Pathology (11)	Prognosis (16)
I	Neuropraxia	Demyelination	Excellent
II	axonotmesis	Demyelination with axonal lost	Good
III	neurotmesis	II + Involvement of endoneurium	Good-fair
IV	neurotmesis	III + Involvement of perineurium	Poor-nil
V	neurotmesis	IV+ Involvement of epineurium	Nil
VI (Mackinnon's modification)	Mixed	Combination of Sunderland's II to IV	Varies

the proximal and distal ends of the zone of trauma (22).

In the distal end, Wallerian degeneration ensues in the next 24-48 hour in which the Schwann cell plays a large part (23). Once the SC sense that the axonal nerves are no longer connected, it will transition from a mature, myelinating type or non-myelinated type (Remak cells) to a proliferative, repair SCs (Bungner SCs) (24) with the mRNA downregulation of myelin-associated proteins (e.g. P0, myelin-associated glycoprotein) (11). The SC also reduce secretion of several proteins such as peripheral myelin protein 22 (PMP22), while increase the production of NGF and BDNF (9). The receptors of neurotrophins (e.g. $p75^{\text{NTR}}$, Trk A, TrkB, GDNF family receptor alpha-1 (GFRA-1), GDNF family receptor alpha-2 (GFRA-2) and cell adhesion molecules, e.g. neural cell adhesion molecule (NCAM), are upregulated (25). Collectively, these are also called regeneration-associated genes (RAGs). These immature SCs will be influenced by the mitogens released from the proximal damaged end and by cellular factors released by other cell types (ie. fibroblasts) as well as blood-derived factors from breakdown of the blood brain barrier (BBB). The SC also release cytokines and recruit macrophages to the site of injury and participate in phagocytosis to clear up axonal and myelin debris within 3 weeks (11). After clearing the myelin debris, the SC will grow on the endoneurial tubes of the extracellular matrix creating the bands of Bungner, which serves as a guidance for nerve regeneration. It have been reported that pericytes, such as SCs, activate during wound healing, inflammation and tissue remodeling (26). These activated pericytes proliferate and follow the lead of angiogenic endothelial cells (26). Indeed, Cattin *et al* showed that hypoxia

after peripheral nerve damage caused macrophages to stabilize Hypoxia-inducible factors 1 alpha (HIF-1 α) and increased Vascular endothelial growth factor-A (VEGF-A) level (27). This induced vascularization, which becomes essential for SC to identify the direction for migration towards the distal stump (27). Interestingly, within the CNS SCs also travels along blood vessels to demyelinated lesions (28). EphrinB3 within CNS myelin prevents SCs from adhering to the myelin, while enhancing them to bind to blood vessels extracellular matrix, especially fibronectin, by upregulating integrin β 1 expression (28).

In the proximal end, axon will undergo “dieback” degeneration, and daughter axons will begin to sprout and elongate from a growth cone (29) through the scars and into the distal end stumps. These are called the “regenerating units”. Since the proximal end is connected to the cell body, its nucleus within the cell body will undergo chromatolysis and move to an eccentric position and upregulate RAGs such as actin and GAP-43 (30). Based on direction signals from local tissue and denervated motor and sensory receptors, the growth cone sends out filopodia to sample the microenvironment and axons elongate (31). Guidance molecules including semaphorins, ephrins, netrins and slits are involved in this process (32). Neurotrophins such as NGF promote nerve regeneration by countering inhibitory regeneration signals such as the collapsin-1 (33). Scar tissue and other debris impede the growth cone from advancing, thus proteases are produced and released to clear its path (34).

Schwann cells and macrophages play a combinatory and reciprocal role in axonal regeneration. Schwann cells produce

NGF, and extracellular matrix components such as fibronectin and laminin which allow the growth cone to make contact (35), while macrophages produce cytokines such as interleukin-1 beta (IL-1 β) to stimulate NGF production by SC (36). This creates a positive feedback loop. Once the growth cone is in contact with an endoneurial tube, it is most likely reaching the end organ or target and complete the regeneration (11).

If the growth cone does not come in contact with a receptor or an endoneurial tube or is impeded by the presence of several intraneural scarring, it will grow in a disorganized manner and produce a painful tissue mass of tangled axons and proliferating connective tissues known as a neuroma (37). The more severe the nerve injury, the more likely growth cones form neuromas due to increased scarring. This reduces the axonal regeneration efficiency (38). Therefore, nerve damage under grade III is considered as good prognosis when there is no endoneurial damage.

Besides the issue of scarring, prolonged axon regeneration can lead to reduction in nerve regeneration abilities. Sulaiman *et al.* have shown that after 6 months, RAGs are downregulated in a rodent model of chronic injury and delayed repair, leading to a reduced capability for nerve regeneration (39). However, recently, it has been reported that treatment with transforming growth factor-beta (TGF- β) plus forskolin significantly increased expression of RAGs in chronic injured nerve and improved axonal regeneration (40, 41).

MICROSURGICAL REPAIR OF NERVE INJURIES

Advancements in illumination and magnification have led to the emergence of microsurgical techniques comprising modern peripheral nerve repair. Contemporary nerve repair entails epineural adaptation of the proximal and distal nerve ends with minimal microsutures. This technique remains the gold standard for severe axonotmesis and neurotmesis injuries. Proximal and distal nerve stumps are correctly oriented prior to suturing and then sealed with fibrin glue. This method of repair is suitable for small nerve gaps below 2 cm in size. A nerve gap is the distance between the proximal and distal nerve stumps following injury and retraction due to elastic properties (42). Some studies have tested sutureless techniques e.g. laser welding which have yet to reach standard surgical practice (43, 44). End-to-end neurorrhaphy (EEN) has limited use since nerve regeneration requires tension-free coaptation to minimize intraneural scarring from postoperative movement. Repairs with tension endangers the vascular supply and increases the likelihood of scar formation (45). As a result, tension-free

repair has become more crucial given the importance of exercise in recovery after nerve injury (46-48). Well-vascularized regions represent another stipulation for neurorrhaphic repairs. An alternative technique is end-to-side neurorrhaphy (ESN), which may be useful in cases where the proximal nerve stump is not obtainable (49). In such circumstances, the distal stump is coapted to the side of the proximal stump. Collateral sprouting is promoted in this technique and restores motor function. However, this technique has multiple drawbacks including the requirement of a donor nerve, minimal sensory recovery, and lower efficacy compared to EEN (50).

NERVE TRANSFERS

Segmental nerve injuries necessitate nerve transfers and are considered the standard for bridging gaps in the peripheral nervous system (51). Nerve transfers coaptate a healthy nerve donor to a denervated nerve. Autografts for peripheral nerve repair are commonly harvested after the injured nerve is exposed from superficial sensory nerves such as sural, saphenous, or the medial and lateral antebrachial cutaneous nerves. Three categories of autografts include trunk, cable, and vascularized nerve grafts (52). Differences in types of grafts produce better outcomes based on their components and the particularities of the injury. Endoscopic techniques have been introduced for nerve harvest. Grafts form a natural conduit that transfers the cellular (Schwann cells) and structural guide for regeneration. Advantages favoring autologous grafts include native tissue that promotes cell adhesion and migration with minimal immunogenicity (53). Autografts have been shown to be superior to epineural neurorrhaphy under tension (54, 55). However, several downsides exist for this treatment. Natural graft procurement results in loss of function and risk of neuroma development at the donor site, multiple grafts required for long nerve gaps, and size and fascicular incompatibility between the injured nerve and graft (56, 57). In contrast, allotransplantation of cadaveric nerve eliminates the loss of function at the donor site as a tradeoff for greater immunogenicity. Allografts require systemic immunosuppression and expose the patient to infection, decreased healing rate and other systemic effects (58). Decellularized and protein-free allografts exist to minimize immune response. In clinical situations where extensive peripheral nerve injuries have been sustained allografts are utilized when donor material is in short supply.

DESIGNING THE “IDEAL” NERVE CONDUITS

Current investments in research and development for periph-

eral nerve regeneration are focused on nerve conduit design. Conduits promote axonal sprouting at the proximal end and guide regeneration towards the injured distal site via chemotactic cues, stem cell seeding, and structural support. The ideal nerve conduit design will incorporate several properties including anisotropically arranged growth factors, enhanced stem cells, biocompatible and electroconductive materials, anatomical specificity, and vascularity.

ROLE OF GROWTH FACTORS

Lately, incorporation of growth factors into the nerve conduit lumen has enhanced the efficacy of conduits (59). Numerous polypeptides emanating from non-neuronal cells following injury promote the growth and guidance for nerve regeneration. The most studied growth factors include NGF and BDNF (60), CNTF, insulin-like growth factor-1 (IGF-1), GDNF, TGF- β 1, FGF-2, GGF, and neuregulins (61-63). Although these factors have been identified in the microenvironment during nerve injury and repair, further research is required to ascertain mechanisms of action. Several problems exist with injecting neurotrophins into wound sites including their short half-life (instability) and the need for high-doses to elicit axonal sprouting (64). Neurotrophic instability means their effect is short-lived which diminishes their utility for long gap nerve injuries that take months to regenerate (64). High dosages of neurotrophins can exaggerate axonal sprouting leading to entrapment and poor recovery outcomes (65). Axons also require topographic guidance cues to reach their distal targets that injections cannot communicate. This makes combining neurotrophic factors with conduit designs more attractive because it can mitigate these tendencies by controlling their release and longitudinal concentration.

Glial growth factor (GGF) is released following nerve injury and reportedly stimulates SC proliferation (66). It promotes signaling exchange between neuronal and glial cells during peripheral nerve regeneration. Studies have incorporated GGF into conduits for defects spanning 2-4 cm resulting in elevated SC numbers, increasing axonal regeneration, and preventing muscle degeneration compared to control (67). Fibroblast growth factor (FGF) is another factor promoting cell growth and regeneration during nerve injury. Studies have incorporated FGF-2 into conduit biomaterial for treating 1 cm defects and reported greater regeneration compared to collagen-based matrices (68).

Schwann cells produce NGF during Wallerian degeneration and promote nerve healing and regeneration (69, 70). When

NGF is applied to poly [LA-co-(Glc-alt-Lys)] (PLGL) scaffolds it activates SC adhesion and prolongs their survival *in vitro*. Modifying NGF concentration or SC seeding in microchannels can both upregulate the overall NGF concentration in conduits (71, 72). Similar to NGF, CNTF is released from SC and nerve stump following injury and enhances sensory and motor neuron survival (72).

STEM CELL ENGINEERING

Stem cell transplantation has an important therapeutic role in stimulating peripheral nerve repair and regeneration. Neural stem cells (NSC) have the capacity to divide, proliferate, and differentiate into multiple lineages *in vitro*. Also, they can produce neurons, astrocytes, Schwann-like cells, and oligodendrocytes (73, 74). Several studies have produced NSCs from a variety of cells including human gingiva-derived mesenchymal stem cells (74), mesenchymal stem cells (75), and induced pluripotent stem cells (76). Differentiation of NSCs into Schwann-like cells have been used for peripheral nerve repair (77). Similar studies reported that NSCs promote axonal regeneration and myelination (78). Additionally, transplantation gingiva-derived mesenchymal stem cells can be induced into pluripotent cells and promote axonal regeneration (79). These findings may be attributed to several cellular features of NSCs including neurotrophic and neuroprotective factor production (BDNF, NGF, FGF, IGF-2, GDNF, etc.) (80-82). Further research is required to ascertain if NSCs generate sufficient levels of functional cells for nerve regeneration. A recent study has implemented CRISPR technology to selectively edit the genome of NSCs to produce both knock-out and knock-in cell lines (83). Selectively editing stem cells may upregulate genes for enhanced stem cell production of neurotrophic factors, tubulins for axiogenesis, or anti-inflammatory factors for longer periods of time (84). Genetically modified NSCs have been shown to accelerate proliferation and differentiation while maintaining the release of neurotrophic factors (85).

Adipose-derived stem cells (ADSC) have been utilized greatly in peripheral nerve regeneration. Several advantages to comprise of ease of harvesting, abundance in supply, non-immunogenicity, and efficacy for nerve regeneration (86, 87). Similar to NSCs, ADSCs likely regenerate nerves via differentiation towards Schwann cells and subsequent release of trophic and neuroprotective factors (88). It is well documented that ADSC-populated conduits outperform empty conduits in several different nerve injuries (88, 89). The downside to using ADSCs is the low risk for teratoma formation after

differentiation into mesenchymal lineage (90). Clinical use of these cells would necessitate the investigation of ADSC spontaneous differentiation.

BIOMECHANICAL DESIGN

Much emphasis has been placed on optimal nerve guidance conduit (NGC) design for promoting nerve regeneration (Fig. 3). This section will review the lineage of NGC design evolution. Hollow cylindrical NGCs were developed to bridge nerve gaps and confine axonal regeneration in a given direction. Developers have made adjustments to materials (natural or synthetic) through various methods including crosslinking, braiding, electrospinning, injection molding, melt extrusion techniques, or combinatorial approaches (91-93). Materials and fabrication methods produce unique mechanical, chemical, and biological properties. An important example involves the generation of electroconductive scaffolds for nerve regeneration. Conductive NGCs can improve peripheral nerve regeneration by increasing the expression of neurotrophic factors and other cellular responses (94). Several studies have demonstrated polypyrrole-based conduits (PPY) comparable to autografts in rat sciatic nerve repair (95). Hollow NGCs have been found to decrease myofibroblast infiltration, scar formation, and collateral axon development (96). The downside to hollow NGCs is their limited capacity for chemotaxis necessary for axonal regeneration towards the distal stump. This results in poor functional recovery when compared to current clinical standards (97). Performance of hollow NGCs worsen as the gap widens generating disorganized axon growth (98).

Figure 3. Extrusion-based 3D printing for NGC. Reproduced from (2).

To improve axonal regeneration, porous NGCs were developed to support components for nerve regeneration such as growth factors, SC, blood vessels, and nutrients (99). The pores are large enough for migration of SC to proceed. Degree of porosity or microchannel size has been tested to exclude

certain components (fibroblasts) and allow more desirable regenerative elements (100). Matrix loaded NGCs have spawned from porous NGCs to regulate the microenvironment of NGC channels. Bioprinted conduits can lay down specified concentrations of components i.e. polysaccharides, proteins, growth factors to provide cues for axonal directionality (101). Matrices or hydrogels accelerate axonal regeneration for longer spans of nerve injury than hollow NGCs or empty porous NGCs (91). Popular hydrogel polymers include hyaluronic acid, fibrin, and chitosan for their ability to direct nerve regeneration (91). The advent of hydrogels has spurred the emergence of anisotropic scaffolds. Anisotropic conduits vary concentrations of hydrogel and cellular components along the length of the conduit to exaggerate the guiding effects of chemical cues. This design feature further enhances axonal alignment and directionality towards the intended target.

VASCULARIZATION

Major transitions during the evolutionary development of vertebrates required overcoming the selective pressure of oxygen and nutritional supplies. Primitive vasculature developed to counteract the metabolic drive and subsequent decline in surface area available for oxygen exchange for the interior cells of emerging multicellular organisms (102, also see John Torday's review in this volume of *Biomedical Reviews*). In the case of nerve regeneration, studies have reinforced the importance of sustained blood flow in nerve regeneration. In acute nerve injuries, hyperemic conditions rather than ischemic conditions is the prevailing event resulting in focal edema (103, 104). Other studies have elucidated the critical role of local microvessels in nerve regeneration by sustaining axonal survival through VEGF signaling and guiding axonal outgrowths to their target (105). Attempts to reinnervate long-standing nerve injuries have reported unreceptive microenvironments for axiogenesis likely due to chronic declines in blood flow (106). Understanding the developmental processes for vascularization is crucial for producing nerve conduits that promote vascular regeneration.

Vasculogenesis is the developmental formation of vascular networks from differentiated endothelial precursor cells (EPCs) or angioblasts. Similarly, angiogenesis denotes the process of capillary growth from preexisting vasculature through sprouting or intussusception mechanisms (107). Finally, arteriogenesis is a distinct process generating small arterioles with low blood flow to larger arteries. Cellular controls driving the sequence of vasculogenesis and adulthood

angiogenesis have been identified in the past decade (108). Growth factors involved in vessel formation include NGF (see 60 and references therein), TGF- β , vascular endothelial growth factor (VEGF), placenta growth factor (PIGF), FGF, platelet-derived growth factor (PDGF), and angiopoietins (107). Comparable to axiogenesis, sprouting vessels require growth factors to sustain development and chemotactic factors to guide sprouts properly (109, 110).

Developing a conduit that promotes peripheral neurovascular regeneration is a major design obstacle. Limited studies are available merging these cellular processes, but studies have reported superior regeneration when nearby vascular bundles are connected to nerve conduits (111). Although nerve regeneration in the conduit group was inferior to the autograft group, electrophysiological and histological examinations reveals comparable findings between the groups in rat and canine models. Incorporating vasculature into conduit design sustained nerve regeneration for 3cm-long-gaps. Development of neurovascular conduits would provide the foundation for regenerating other tissue types (dermis and muscle) in medical conditions.

ACCELERATION OF AXONAL REGENERATION THROUGH CONDUITS – ROLE OF FUNCTIONAL ELECTRICAL STIMULATION

A growing trend for nerve regeneration is the implementation of electrical stimulation (ES). As previously mentioned, ES and electroconductive conduits are effective tools for enhancing reinnervation following nerve transection. ES was found to accelerate the rate of axonal outgrowth in multiple studies (94). Furthermore, the majority of ES benefits can be derived from stimulation for 1 hour at 20Hz (112). Gene expression is altered during ES that upregulates growth factor production, tubulin transport, and axonal elongation (113). Experiments have also found that ES promotes nerve regeneration despite a delay in nerve repair following injury in both rats and humans (30, 114). Incorporating the effects of ES into electroconductive conduit design should extend our capacity for spanning longer nerve injury gaps.

CONFLICT OF INTERESTS

There are no conflicts of interests to disclose in the context.

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