Scaffolds for bridging sciatic nerve gaps

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36.1 Overview

Peripheral nerve injury (PNI) is a common affliction for which there are few effective treatment options. PNI, including root avulsions, occurs in 2%–5% of all trauma cases in the United States, including assaults and motor vehicle accidents, and a significant proportion of warfighter injuries involve peripheral nerves [2–4]. PNIs that do not result in damage to overall nerve structure, such as crush or stretch injuries, generally result in a wait-and-see approach to determine if function returns spontaneously [5–7]. However, PNI resulting from a nerve transection requires a surgical procedure to reconnect the proximal and distal nerve stumps directly (if possible) or by inserting a biological or synthetic bridging graft between them [4]. Overall, in cases requiring surgical intervention, outcomes of surgical repair for traumatic PNI are generally unsatisfactory as only 50% of patients achieve good to normal restoration of function, irrespective of repair strategy or injury location [9].

Tissue damage to peripheral nerves can result from both traumatic injuries and iatrogenic injuries occurring during surgery. Extensive surveys indicate that the radial and ulnar nerves are most prone to trauma in the upper limbs, whereas sciatic and peroneal injuries are most common in the lower limb [10]. PNIs are often associated with fractures of nearby bones and central nervous system (CNS) damage thereby compounding the disability and impeding proper diagnosis of peripheral nerve damage. In general, PNI is characterized by flaccid atrophy of surrounding muscles leading to partial or complete paralysis. Further sensory loss, including proprioception in the skin areas at the distal end, is also observed. Overall, PNI can result in severe and debilitating motor and sensory deficits, greatly affecting the quality of life of afflicted patients.

As noted, the most significant challenge is attaining meaningful functional recovery in cases requiring the surgical repair of severed nerves. In these severe injuries, the

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axonal segments distal to the transection site undergo Wallerian degeneration, in what is believed to be an inevitable consequence of having been disconnected from its neuronal cell body that resides within or adjacent to the spinal cord for motor and sensory axons, respectively. In these cases, regenerating axons must not only regrow across any gap that persists between the proximal and distal stumps but must also traverse the entire length of the distal nerve to reach appropriate targets. In humans, at average rates of axon regeneration of 1 mm per day (approximately, 1 inch per month), it can take on the order of several months to over 1 year for axons to reach distal targets following PNIs in the upper arm or upper leg. This slow rate of axonal regeneration coupled with chronic denervation of muscle–causing diminished capacity for functional restoration over time–results in prolonged and often permanent functional deficits.

Thus, there is a critical unmet need for repair strategies capable of accelerating this regenerative process. As noted above, after PNI involving nerve transection, the method of surgical repair primarily depends on the severity of the injury. In cases of relatively small segmental defects, the desired surgical repair strategy is direct reanastomosis whereby the proximal and distal nerve stumps are directly sutured together end-to-end, provided that the repaired nerve will be left tension-free. However, cases where there is a significant segmental defect (e.g., centimeter scale) require a bridging graft to act as a guide and protective encasement for regenerating axons. Bridging strategies include sensory nerve autografts, decellularized nerve allografts, and biological or synthetic nerve guidance tubes (NGTs). The current so-called "gold standard" bridging strategy is the nerve autograft, generally a sensory nerve–taken elsewhere from the same patient–that is deemed less critical to patient function than the (generally) motor nerve to be repaired. Standard nerve autografts are typically used for gaps of 1–10 cm, with an effort to utilize vascularized nerve autografts for gaps >10 + cm [11–13]. Moreover, the use of autografts is hindered by limited availability of donor nerve, the need to perform a second surgical procedure, and donor site morbidity, including the deliberate infliction of a sensory deficit by removing an otherwise uninjured nerve [14]. In addition, there can be inefficient functional regeneration arising due to a mismatched nerve diameter, differences in fascicular number and distribution, and modality inhibition when a sensory nerve is used to repair a motor nerve [14]. Additionally, potential complications following autograft harvest include risk of infection and formation of painful neuroma. These issues have motivated the field to try to develop alternative bridging strategies that are capable of meeting or, ideally, exceeding the performance of the autograft. Alternative approaches to nerve autograft have included attempts to bridge severed nerve endings with allografts (nerve tissue from another individual, typically cadaveric) or even xenografts (animal derived). However, this presents limitations including immune rejection, risk of disease transmission, secondary infection, and limited supply. Moreover, NGTs are only effective for relatively short gaps (<1 cm) and are generally only used for repair of noncritical sensory nerves that are close to end target (e.g., nerves in hand or foot). Unfortunately, although there have been notable advancements over the last few decades, there has been limited success to develop a suitable solution to replace the autograft.

Overall, despite best efforts and modern surgical techniques, functional restoration is often incomplete [15]. This underscores the clear and compelling need

for biomedical research to develop novel strategies and grafting options to improve outcomes following nerve damage [4,16]. Indeed, biomaterial scientists and tissue engineers have pioneered the development and fabrication of natural and synthetic polymer-based scaffolds consisting of varying architecture that can be implanted as conduits to bridge a nerve gap. Next-generation engineered nerve conduits or nerve guides possess mechanistically inspired biological activity resulting in an ability to enhance regenerative processes (e.g., host Schwann cell infiltration and axonal regeneration) by providing haptotactic, chemotactic, and topographic guidance. Additionally, nerve conduits ensure localization of neurotrophic factors (endogenously secreted by host cells and/or added exogenously) and prevent undesirable cellular invasion (e.g., fibroblasts) that could form scar tissue obstructing regeneration across the nerve gap. A particularly promising tissue engineering approach involves the development of "living scaffolds," which are regenerative bioscaffolds composed of living neural cells in a preformed, often anisotropic, three-dimensional (3D) architecture. The objective of these living cellular-biomaterial scaffolds is to (1) motivate and direct guidance of host axons and (2) facilitate migration and organization of host cells (e.g., Schwann cells). The most common objective of next-generation bioscaffolds is to facilitate the regrowth of axonal tracts damaged from disease or injury by mimicking crucial aspects of developmental pathfinding.

In this chapter, we provide an overview of the current state-of-the-art for repair of trauma to the peripheral nervous system (PNS) while detailing efforts to develop alternative scaffold-based bridging strategies to facilitate regeneration across segmental nerve defects. A focus is given to next-generation strategies that have been or are currently being evaluated in the sciatic nerve injury (SNI) model. Indeed, the SNI model remains by far the most prevalent animal model for studying neuroregeneration and for developing technologies to bridge large nerve gaps. This chapter, therefore, provides an overview of sciatic nerve anatomy and function. This chapter also reviews biomaterial-based strategies for bridging sciatic nerve gaps in context with key neurobiological mechanisms involved in axon pathfinding that should be considered while engineering next-generation nerve conduits. On this front, several promising emerging strategies exist for the development of living regenerative scaffolds consisting of aligned glial cells and/or longitudinal axonal tracts that have driven robust and targeted axonal regrowth and neural cell migration. Finally, we discuss the advantages, challenges, and future potential of engineered bioactive materials and/or living scaffolds in PNI repair, regeneration, and functional recovery.

36.2 Peripheral nervous system

36.2.1 Nervous system anatomy and function

The nervous system can be anatomically and functionally divided into the CNS and PNS. The CNS, relying on a specific macro and microenvironment for function, is anatomically protected by the bony skull and spinal column. Anatomically, the CNS is composed of the brain and spinal cord. The CNS functions in the production and transmission of efferent signals and the interpretation of incoming afferent signals from the PNS.

On a cellular level, the nervous system can be divided into the primary functional cells, called neurons, and the predominant support cells, known as glia. Neurons are composed of a cell body, dendritic processes responsible for processing incoming information, and an axon that transmits information to downstream neurons or end organs. There are several subtypes of neurons that are generally classified by anatomical region, neurotransmitter type, or based on inhibitory versus excitatory function. The roles of glia are quite diverse and crucial for nervous system function. The main glial cell types are astrocytes, microglia, oligodendrocytes, ependymal cells, radial glia, and satellite cells in the CNS, and Schwann cells and satellite cells in the PNS. Glial cells are responsible for maintaining the microenvironment of the nervous system. Of interest is the spinal cord, where motor and sensory neurons are located that project axons as the basis for peripheral nerves. Outgoing efferent motor neurons are found in the ventral horn of the spinal cord gray matter, whereas afferent sensory dorsal root ganglia (DRG) are found all along the dorsal sides of the spinal cord, with long axon tracks extending up to 1 m (in humans) to innervate the body. The PNS is composed of sensory and motor axon tracts. These axon tracts are supported by Schwann cells and satellite cells, responsible for the maintenance of ionic gradients and the production and maintenance of insulating myelin sheaths around individual axons [8]. Schwann cells also play a key role in regeneration, extracellular matrix (ECM) production, and signaling [8,17]. Peripheral nerves are in effect bundles of axonal projections carrying both efferent and afferent information.

Nerves in the PNS are encapsulated in several layers of tissue that serve to protect and increase the efficiency of the nervous system. As a peripheral nerve exits the spinal canal, the dura and arachnoid mater (outer layers of the CNS) come together to form the outer layer of nerve sheathing, known as the epineurium. Each individual myelinated nerve axon is covered in endoneurium, with axon bundles held together by perineurium. Several bundles and associated vasculature constitute a nerve held together by the thicker fibrous epineurium [8] (Fig. 36.1).

36.2.2 Sciatic nerve anatomy and function

In particular, the sciatic nerve has been a focus of PNI research efforts, mainly due to its ease of accessibility. The sciatic nerve is the longest peripheral nerve in the body and is a mixed motor-sensory nerve. In humans, it runs from the lower spinal cord, into the buttocks, down the back side of the legs, and into the foot [18]. The rat sciatic nerve is the most commonly used model for studying PNIs. In the rat, the sciatic nerve stems from L4-L6 in the spinal cord, where it is unifascicular; 5–7 mm distal to the trochanter (where the muscle and bone attach), the nerve splits into 2 fascicles and then 4 fascicles [19]. The tibial portion of the nerve branches off into the tibial (predominantly motor, with some sensory fibers) and sural (sensory) nerves, whereas the peroneal portion of the nerve branches off into the peroneal nerve (mixed motor-sensory) and a cutaneous nerve branch that joins into the hamstring muscle [19,20] (Fig. 36.2).

Thus, the nervous system relies on a highly complex microarchitecture and cell interaction schema to regulate the complex function of the human body. While still an area of intense study and development, the current understanding of the nervous system has

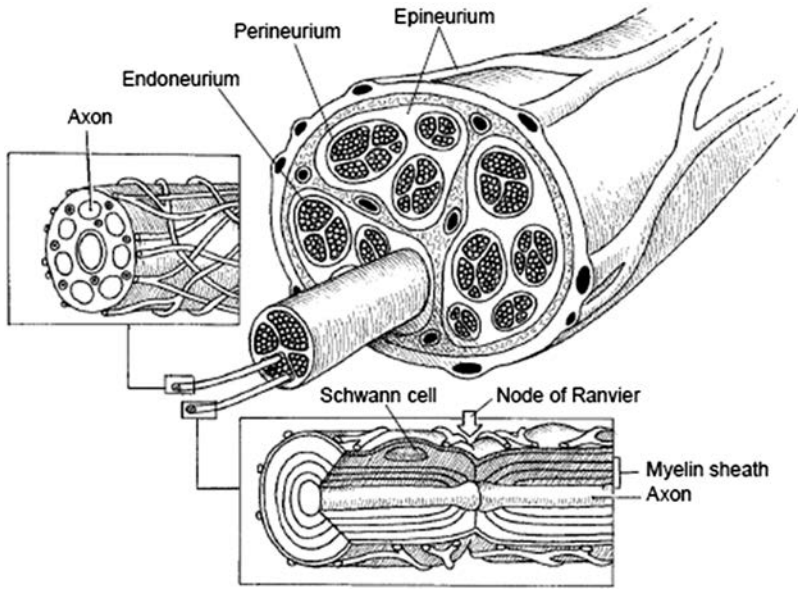

Figure 36.1 Anatomy of a nerve. Nerves consist of a modal structure, composed of an axon that is surrounded by a membrane called the endoneurium. Several of these axons comprise a fascicle, which is bounded by the perineurium. In turn, numerous fascicles make up the peripheral nerve, which is covered by the epineurium. The inset on the left shows an unmyelinated axon and the inset on the bottom shows a myelinated axon.
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propelled groundbreaking research spanning basic neuroscience, neural engineering, and clinical neurology and neurosurgery. Moreover, knowledge about developmental biology informs and inspires efforts to facilitate nervous system regeneration after injury. Indeed, the proper development of the nervous system relies on precisely controlled cellular and axonal guidance, whereas sustenance of normal function of the nervous system is based on the continued maintenance of these complex networks. Regenerative processes build off of these developmental and homeostatic mechanisms and must be clearly understood to augment repair and improve functional recovery following trauma.

36.3 Axon pathfinding during peripheral nervous system development

36.3.1 Biological scaffolds provide guidance cues for axon pathfinding

Throughout embryogenesis and prenatal development, there are many instances in which neuronal migration and axonal pathfinding are mediated by preexisting cells

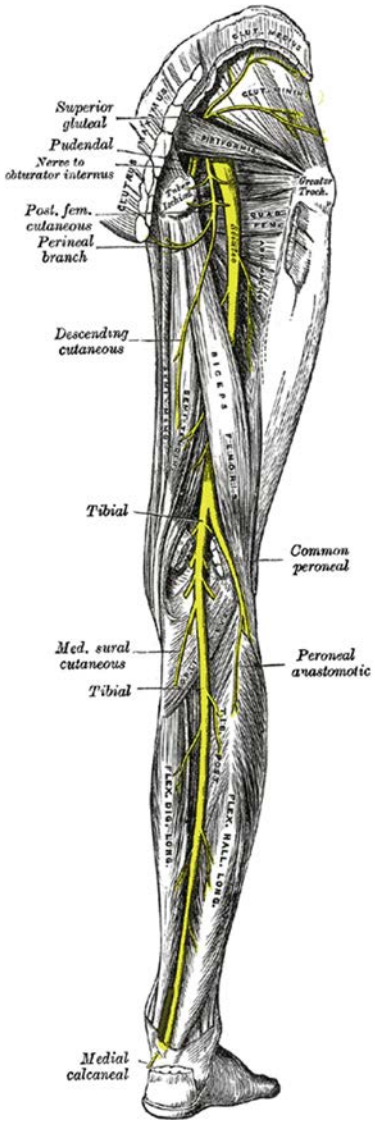

Figure 36.2 Sciatic nerve anatomy. The sciatic nerve originates near the pelvis and winds down along the posterior side of the leg, branching into the tibial nerve, common peroneal, and sural nerves. Reproduced from H. Gray, H. Gray, Anatomy of the Human Body, Philadelphia, Lea & Febiger, 1918 (Online edition Bartleby.com, 2000).

and processes. For example, axons frequently extend along preexisting axons or basal lamina to reach and innervate their respective targets [21–24]. In a developing fetus, peripheral glial and other cells direct pathfinding of axons in the transition zone between the PNS and CNS; these cells can also be referred to as guidepost cells. Seminal studies have shown that during development, peripheral glia formed

funnel-shaped arrays that guided pioneering motor axons into the periphery. They observed that the axons made typical growth cone contact along the glia, and ablation of these guidance cells caused disruption of axon extension in drosophila and grasshopper models [23,25]. Thus, the guidepost cells and peripheral glia functioned as a substrate and guidance cues to prepattern the transition zone for correct routing of axons between the CNS and PNS during development [23]. This mechanism is seen to be preserved in mammals [26].

Pioneering axons themselves also serve as scaffolds for the guidance of subsequent axons in the nervous system. Pioneer axons reach the end target first, and follower axons use them as a guide to the specific end target. For example, it has been found that in the PNS, pioneering axons are responsible for forming the initial pathways that subsequently become nerves in the antenna and legs of grasshoppers [27]. Additionally, studies have found that motor axons generally serve as pioneer axons, with sensory axons or other motor axons being guided by them to reach the correct end target [28]. Overall, these examples demonstrate the developmental basis for a subset of contemporary tissue-engineered scaffolds being used for neuroregeneration.

36.3.2 Mechanisms of axon guidance

To develop successful tissue-engineered scaffolds for neuroregeneration, the mechanisms responsible for neural cell migration and axon guidance must be understood and recapitulated. Both cell motility and axon guidance have been shown to occur through one or more of the following mechanisms: (1) contact signaling via structural cues presented along cells and/or on ECM; (2) gradients created by diffusion of cell-secreted soluble factors; and (3) substrate mechanical and geometric properties. Of note, living scaffolds possess the ability for "juxtacrine" signaling based on the simultaneous and often synergistic presentation of the aforementioned cues [29–34].

36.3.2.1 Haptotaxis: contact dependent signaling

A combination of attractive and repulsive structural cues presented to growth cones direct axons to their targets. These haptotactic proteins appear both directly on cell surfaces and throughout ECM complexes and serve as guideposts during development and regeneration [21,35]. Prominent structural cell contact cues involved in axon guidance include cell adhesion molecules (CAMs), including L1-CAM and NCAM (as with neural cell migration), among others [21,35]. L1-CAM is a homophilic binding transmembrane protein, whereas NCAM is a homophilic binding glycoprotein that is present on the surface of cells. These proteins play a key role during development and are involved in axon fasciculation and neurite outgrowth. CAMs have been shown to affect glial activity and axonal outgrowth in both in vitro and in vivo models. Axons growing in vitro have shown a preference for specific CAMs patterned onto a substrate, with distal axons (greater than 55 μm from the cell body) selectively following L1-CAM patterning and proximal axons recognizing both L1-CAM and N-cadherin [36].

Additionally, other types of CAMs regulate axon guidance by serving as attractive or repulsive cues. Most contact-dependent molecules serve as repulsive cues to guide axons, among them are several classes of semaphorins, slit, netrins, Robo, and Eph/ephrin proteins. We will focus on Eph receptor and ephrin ligands as they are the largest class of tyrosine kinase receptor transmembrane proteins that are capable of bidirectional signaling, leading to attractive and repulsive behavior. For a review of other guidance molecules, please refer to the following reviews [37–41]. Eph receptors and ephrin ligands work in concert to direct cell adhesion, specifically axon guidance and cell migration in the developing nervous system as well as into adulthood [42]. Eph receptors are a subtype of receptor tyrosine kinases that consist of a hydrophobic transmembrane domain and an N-terminal extracellular binding domain that interacts with the ephrin ligand. There are two types of Eph receptors and ephrin ligands—A and B. The ligand ephrin A is tethered to the membrane via glycosylphosphatidylinositol linkage, whereas ephrin B is a transmembrane ligand. Interestingly, the Eph/ephrin signaling cascade is bidirectional and can be initiated either by the receptor or ligand. Eph receptor activated signaling is referred to as forward signaling and results in repulsion, whereas ephrin ligand activated signaling is termed reverse signaling and leads to attraction [42,43]. For example, as discussed, motor axons reach their muscle end targets first and act as pioneer axons along which sensory axons are guided. This is believed to be directed by contact-dependent cues, including Eph/ephrin A signaling, where EphA receptors on motor neurons engage with related ephrin A ligands on sensory axon growth cones to direct axon–axon guidance [28].

36.3.2.2 Chemotaxis: soluble factor signaling

Following injury to peripheral nerves, Wallerian degeneration occurs at the distal end of the injury. Simultaneously, Schwann cells activate and macrophages rush into the site of injury to clean up debris. Schwann cells secrete soluble factors that promote neuron survival, stimulate axon outgrowth, and guide axons for nerve regeneration. Such factors include nerve growth factor (NGF), brain-derived neurotrophic factor, fibroblast growth factor (FGF), glial-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), and insulin growth factor, among others [38]. In response to Schwann cell secretion of these factors, expression of receptors for these factors on regenerating axons is modulated to maintain an optimal proregenerative environment. For example, expression of GDNF and its receptors, as well as CNTF receptors, has been seen to be upregulated following injury in the sciatic nerve and other PNI models [44].

36.3.2.3 Mechanotaxis: physical/geometric influences

In addition to conventional chemotactic and haptotactic cues for axonal guidance, it is becoming well established that the mechanical and physical properties of a cell's environment has a significant impact on cell growth and behavior [34,45]. The effects of microenvironmental physical properties on neurite outgrowth may be referred to as "mechanotaxis." Prior work has shown that parameters of neurite outgrowth such as growth rate and neurite branching depend on matrix mechanical properties (in 3D and

2D) [46,47]. This includes substrate stiffness, pore size, substrate size and curvature, and mechanical stimuli, to name a few [34,46–49]. Specifically, it has been found that neurons prefer to grow on soft substrates (with modulus similar to the brain), along substrates of curvature similar to axons in vivo, on rougher edges, and respond well to moderate mechanical stimuli [34,45–51].

36.4 The challenges to nervous system repair and regeneration

A variety of insults can lead to neuronal and glial cell loss, including traumatic injury and neurodegenerative diseases. In addition, disconnection of axonal pathways is a common feature across multiple types of neurotrauma and neurodegenerative disorders. Following trauma to the PNS, tissue and axon regeneration is generally more successful than that found following CNS injury, owing primarily to the proregenerative response of resident Schwann cells; however, functional restoration following major nerve lesions (e.g., several centimeters or greater in length, peri-midline PNI necessitating ultralong regenerative distances for axons) is generally poor due to insufficient axonal reinnervation of distal targets [4]. To date, neither exogenous cell replacement strategies nor acellular biomaterial-based approaches have been successful in orchestrating neural tissue formation and long-distance axonal pathfinding in the nervous system.

36.4.1 Intrinsic nerve regeneration following trauma

Immediately following nerve injury, a process known as Wallerian degeneration occurs, where axonal segments undergo a controlled breakdown and macrophages and glial cells migrate into the site of injury to clear away debris and prepare the environment for regeneration. The myelin and axons degenerate, leaving behind Schwann cells in a basal lamina tube that surrounded original axon fiber. Axons begin to regenerate within a few hours of axotomy, sprouting through the now exposed node of Ranvier. The cut tip of the axon swells with endoplasmic reticulum, mitochondria, and microtubules. The regenerating sprouts grow down the bands of Bungner, which are longitudinally aligned Schwann cells that form post injury, with one side of the growth cone in contact with the Schwann cell basal lamina and the other side in contact with the Schwann cell membrane. Over the months following nerve repair, the nerve grows in diameter and many of the branches that sprouted in the initial stages of regeneration disappear [52]. The exact outcome is dependent on connection with an end target. Remyelination of regenerated axons starts after approximately 8 days. Remyelinated axons are enveloped by Schwann cells in the basal lamina tube, which then wrap around them and form myelin [52,53]. Interestingly, the distance between nodes is smaller than uninjured axons, likely due to the lack of stretch growth that the axons experience after regeneration as compared to development [52]. Because of the smaller internodal distance and thin myelin sheath, nerve conduction velocities

are seen to be lower in regenerated nerves. Although axons are directly involved in the regenerative process, the neuronal cell body is undergoing many changes as well. Regeneration is associated with increased expression of genes and proteins that are associated with axon growth during development. These include gap-associated proteins, tubulin, and actin [52].

36.4.2 Current clinical approaches for nerve repair

It is largely accepted that immediate primary repair of a lesioned nerve, consisting of direct reanastomosis, results in increased regeneration and functional recovery [54]. Here, the epineurium of the proximal and distal ends of the damaged nerve is sutured together. Although it was long believed that the repair should be devoid of tension on the nerve, advancement in the last few decades show that a direct repair under modest tension can yield acceptable recovery [55]. For large defects, such as in cases where performing a primary repair would lead to undue tension, the current gold standard for surgical repair is an autologous nerve graft (autograft). There are three types of autografts [8]: (1) cable grafts—several smaller nerve grafts aligned in parallel to mimic fascicles; (2) trunk grafts—mixed motor-sensory whole-nerve grafts; and (3) vascularized nerve grafts—whole nerve graft including well-vascularized bed. The most common source for the autograft is the sural nerve, a sensory nerve in the calf region of the leg, due to its ease of accessibility, relatively large diameter, and because it is relatively dispensable due to it being a purely sensory nerve. Nerve grafting involves transecting the nerve ends to excise the area of injury and suturing in a graft nerve that is approximately 10%–20% longer than the gap length because fibrosis of the connective tissue results in shortening of the autograft length over time [8]. The graft is typically reversed (i.e., flipped 180°), to decrease chances of aberrant axon outgrowth into any remaining branches of the autograft, and sutured into the site of injury without tension [8].

Although autografts have only been shown to promote adequate functional recovery in 50% of patients, this is currently higher than other treatment options. Some of the concerns surrounding the use of autografts have been addressed by the use of allografts, where nerves from another subject are used to repair nerve defects in the patient. However, allografts remained largely ineffective due to the immunogenic host response. The cellular components of the allografts elicited an increase in immune cells in the injured area. Several techniques have been implemented to decellularize allografts. Some remain ineffective due to the production of debris or lack of structure of the allograft, leading to impaired neurite outgrowth. However, if allografts are decellularized appropriately, functional outcome is believed to be improved, but not equal to those attained by autografts. Other approaches include implementation of a nerve guidance conduit composed of nonorganic polymers and/or biologically inspired materials, such as collagen, to help regenerating axons reach their end target. This will be discussed further below.

As mentioned, functional recovery in patients treated with autografts is typically unsatisfactory. Although this has many causes such as long gap lengths, long total regenerative distances, and delayed repair scenarios, one important consideration

is that in clinical practice autografts are virtually always acquired from a sensory nerve, whereas the nerve being repaired is generally predominantly motor, or in the case of the sciatic nerve, a mixed motor-sensory nerve [56]. Several studies have concluded that regenerating motor axons are preferentially guided by motor Schwann cell basal lamina to the end target, analogous to how growing motor axons are preferentially guided by motor pioneer axons during development, as previously described. In the presence of sensory Schwann cell basal lamina, motor axons are believed to be selectively pruned, again analogous to how growing motor axons are repulsed by pioneer sensory axons during development [57]. A study by Nichols et al. found that transplantation of a 5 mm sensory nerve graft into a mixed nerve in rats resulted in significantly decreased total number of fibers, fiber density, percent nerve, and axon regeneration distal to the injury 3 weeks after injury when compared with motor and motor-sensory nerve grafts for repair [57]. Because it is generally not feasible to use a healthy motor or mixed-modality nerve to treat a defect clinically in humans, there is a need to develop modality-specific grafts that can effectively promote functional nerve regeneration.

36.5 The sciatic nerve injury model for peripheral nerve repair in rats

36.5.1 Sciatic nerve injury overview

Preclinical experiments in neuroregeneration research have mainly been carried out in animal models because, to date, in vitro investigation of nerve regeneration is limited due to the structural complexity of the PNS, which is difficult to reproduce in vitro [58]. The most commonly used experimental paradigm for the preclinical investigation of peripheral nerve regeneration is the SNI model in rats or mice. Among the various reasons that contribute to the widespread use of SNI, some of the most important are (1) the large size of the sciatic nerve which facilitates surgery; (2) the easy surgical access; (3) data that are directly comparable with previous studies, because a large majority of have been carried out using the SNI model; and (4) SNI leads to significant motor deficit, and functional regeneration can be monitored through electrophysiology and gait analysis.

The sciatic nerve is widely accepted as the primary model used to study PNI repair and regeneration. The rat or mouse sciatic nerve is most commonly used, the nerve is transected, and various repair strategies are implemented by researchers to study the effects on nerve regeneration. This model is also used to study neuropathic pain and functional recovery following PNI. To this end, several outcomes may be assessed to determine extent of recovery, including animal behavior over time following injury and/or repair; histomorphometric analysis of the nerve both within the repair site and distal to the repair site; histomorphometric analysis of the (de- and re-) innervated muscle; and electrophysiological response of the nerve and muscle as measured by compound nerve action potentials (CNAPs) and compound muscle action potentials (CMAPs) [59,60].

36.5.2 Surgical considerations of using sciatic nerve injury model

Experimental surgery on the sciatic nerve is relatively easy due to its large size (the largest nerve in mammals). The sciatic nerve is a mixed nerve which originates from the lumbosacral plexus and ends at the knee level with its terminal division that is usually represented by a trifurcation: the tibial nerve (the largest of the branches), the common peroneal nerve, and the sural nerve [61]. However, there is high anatomical variability in the number and site of origin of sciatic nerve terminal branches that should always be taken into consideration, especially in the identification of the lesion site. A second methodological consideration about surgery is the maximum length of the sciatic nerve defect that, in the rat, is generally limited to be 2.0 cm or less. Although the bridging of gaps longer than 2.0 cm has been described in the rat [62], it is generally preferable to move to larger animal models (e.g., rabbits, sheep, or pigs) to test regeneration across long segmental defects in vivo.

36.5.3 Functional assessment in sciatic nerve injury model

Although the assessment of behavioral outcomes is generally the most important evaluation parameter from a preclinical perspective, most currently available methods for measuring functional recovery after SNI are characterized by a high degree of variability which, unfortunately, limits data interpretation. Regarding motor functional recovery, the most commonly used test is the calculation of the sciatic functional index [63,64]. Although this method is very popular in peripheral nerve regeneration research, its validity has been questioned [65]. Therefore, more recently, the availability of high-performing video cameras has allowed the development of more reliable computerized gait analysis systems based on video recording of the animals [66,67].

36.5.4 Electrophysiological assessment in sciatic nerve injury model

The electrophysiological assessment of nerve and muscle recovery is a predictor of nerve regeneration that is closest to the direct assessment of motor or sensory function. Being a mixed nerve, the electrophysiological assessment of the sciatic nerve can be carried out both for the efferent and afferent components. Because recovery of motor function is the most relevant postoperative achievement that is sought in preclinical models, the most used electrophysiological method is the recording of evoked CMAPs after electrical stimulation proximal and distal to the lesion site [68,69].

36.5.5 In vivo imaging in sciatic nerve injury model

Recent advances in in vivo imaging techniques of tissues and organs have expanded their use to small animal species. As regard to SNI investigation, two methods are receiving growing interest for monitoring the nerve regeneration process in vivo: ultrasonography and magnetic resonance imaging (MRI). Although these modalities are not able to directly track axon regeneration, surrogate markers for regenerative processes may be observed such as nerve continuity, anisotropy, blood flow

(revascularization), and swelling/inflammation, among other parameters. Ultrasound imaging has the advantage that it can be obtained using relatively cheap instruments. This technique can thus be used in rat sciatic nerve to monitor the progression of nerve tissue regeneration, e.g., within the nerve grant and/or conduit [70]. MRI requires much more expensive devices that should be adapted to the size of the animal species under investigation [71]. However, if a dedicated facility is available, MRI holds great potential for in vivo investigation in experimental SNI models [72,73].

36.6 Biomaterial and bioactive approaches for nerve regeneration

36.6.1 Nerve guidance tubes

Clinically, NGTs are generally employed where a portion of a nerve is missing or must be excised due to extensive damage. NGTs are used to bridge the gap between the proximal and distal ends of a severed nerve. NGTs serve to guide axonal projections in a more organized fashion and prevent extraneural extension of growing axons [74]. Classically, synthetic materials have been implemented for nerve guidance and protection in PNS surgery, such as silicon tubes [74]. However, the use of silicon alone proved problematic due to a fibrotic host response, poor nutrient transfer, and the need to invasively remove the scarred tissue later. NGTs manufactured from other synthetic materials such as poly(glycolic-acid) (e.g., NeuroTube, Synovis, St. Paul, MN, USA), polylactic acid, and poly(lactic-co-glycolic acid) have provided a solution to problems inherent to silicon NGTs, for instance, by improving mass transport via porosity and controlled degradation. Biologically based NGTs have also been introduced to market, such as NeuraGen from Integra (Plainsboro, NJ, USA) and NeuroFlex from Stryker (Kalamazoo, MI, USA), both composed primarily of cross-linked collagen type I. These NGTs are suggested to allow improved nutrient and neuroactive substance exchange [75]. In addition to collagen tubes, NGTs made from rolled fibronectin mats have also been investigated. Nerve wraps are a similar product, consisting of a tube that is open longitudinally, and can therefore be placed around a damaged nerve that remains otherwise structurally intact. Although some products like Neuromend (Stryker) are type I collagen, others are synthesized from biologic tissues directly, for instance, Axoguard (Axogen, Alachua, FL, USA) is manufactured from ECM derived from porcine intestinal submucosa. These NGTs and wraps are rigid enough to provide some protection of the repaired nerve from mechanical compression, while retaining flexibility to move with natural limb movement. All of these aforementioned products are fully absorbable over a time frame of 3–6 months. These NGTs and nerve wraps are available in several different configurations and diameters.

36.6.2 Acellular nerve allografts

Another technique for peripheral nerve repair utilizes acellular grafts produced from donor peripheral nerves, referred to as acellular nerve allografts (ANAs). Although various techniques to create ANAs have been applied, in general the allografts are

prepared through a decellularization process, such as lyophilization, irradiation, or detergent processing, among other means. This allows preservation of the underlying ECM structure of the nerve to allow for effective host cell infiltration, axonal guidance, and structural support [76,77], while removing donor cellular components that would elicit a host immune response. The Avance graft from Axogen (Chalahua, FL, USA) is an example of a commercially available ANA manufactured using a proprietary decellularization process and is available in multiple lengths for convenient grafting. In both experimental models and pooled clinical analysis, ANAs have been found to perform better than NGTs alone and, in some select instances, have approached the efficacy of the autograft [78,79]. A proposed mechanism for the observed superiority of ANAs to NGTs is attributed to the endoneurial microstructure that may enhance cell infiltration and therefore increase host axon growth across the graft [80]. Currently, several groups are investigating approaches combining ANAs with cellular and growth factor delivery [81–83]. With further studies needed for optimization, ANAs remain an attractive alternative to autografts.

36.6.3 Incorporation of extracellular matrix proteins in nerve guidance tubes

In an effort to promote successful regeneration by more closely mimicking the natural ECM, researchers have combined several ECM-derived molecules within NGTs. One such example is implantation of a silicone NGT filled with collagen, laminin, and fibronectin into a rat PNI model. Here, a majority of the animals treated with laminin-fibronectin exhibited regeneration that bridged the nerve lesion and increased nerve diameter and myelination [84]. In another study, collagen I NGTs filled with laminin- and fibronectin-coated collagen fibers were transplanted into a PNI model. Post transplantation, an increase in the number and myelination of regenerating nerve fibers was seen in the group treated with the fibronectin-coated collagen fibers, as compared to the control groups consisting of uncoated collagen fibers [85]. Additionally, an NGT composed of collagen I and poly(ε-caprolactone) (collagen/PCL) was found to elicit electrophysiological outcomes and muscle innervation that was similar to that of autografts following repair of sciatic nerve gaps in rats [86]. Collectively, these results suggest that NGTs containing ECM constituents may be more effective in nerve regeneration than empty NGTs alone. As an example, a novel commercially available construct on the market, Nerbridge (Toyobo, Osaka, Japan), is an NGT composed of a PGA tube filled with an inner collagen matrix with microtube guidance channels, intended to improve nerve growth and promote angiogenesis.

In addition to hollow tubes functionalized with ECM proteins or growth factors, advancements in conduit-lumen structure have been made. Recently, many groups have enhanced traditional NGTs by implementing aligned or oriented nanofibers through techniques such as electrospinning, functionalizing the conduits with tethered gels or microspheres, and incorporating structured channels to promote axon guidance. These include electrically and magnetically aligned matrices, micropatterned surfaces, gradients of neurotrophic factors, and fibers containing longitudinal grooves [33,87–89] (Fig 36.3). The parameters of the NGTs themselves have been manipulated extensively,

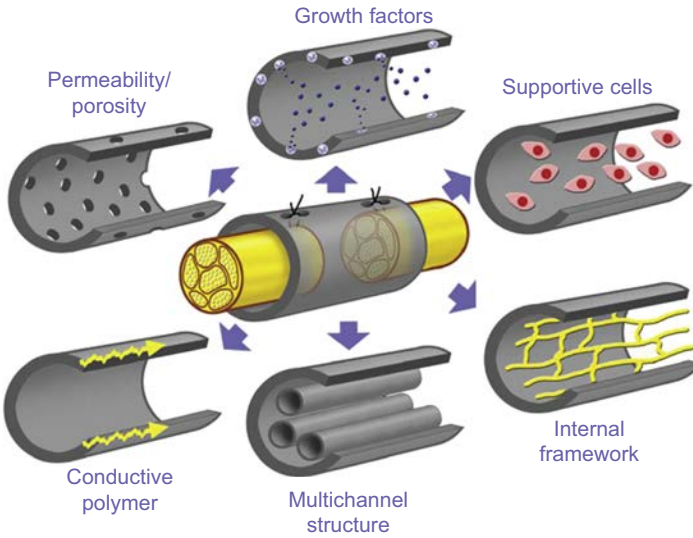

Figure 36.3 Modified nerve guidance tubes. Conventional hollow nerve tubes are modified by optimizing porosity, inclusion of growth factors, and supportive cells, incorporating an internal framework and multichannel structure, or using a conductive polymer to enhance nerve outgrowth and regeneration.
Reproduced with permission from G.C. de Ruiter, et al., Designing ideal conduits for peripheral nerve repair, Neurosurg Focus 26 (2) (2009) E5.

with different thickness, mono- or multichannel structure, and tube wall porosity. Although current biomaterial-based conduits effectively mitigate problems inherent to earlier generation implantable products (which exhibited a high incidence of scarring and fibrosis, acting as major obstacles for nerve regeneration), they do not yet perform near the level of the autograft, especially for longer defects (>1.5–2.0 cm) [90].

36.6.4 Incorporation of soluble factors

The effects of chemotactic cues on axon guidance have been studied extensively. Multiple studies have shown that incorporating these factors into nerve conduits either individually or in combination significantly enhances nerve regeneration [30–33]. Addition of neurotrophic factors into nerve conduits was shown to increase regenerating axon density, as well as Schwann cell migration, alignment, and eventual myelination at the injury site [32,33]. The spatial presentation and gradient of both soluble factors and membrane-bound structural cues play a significant role in axon guidance. For instance, it was reported that axons extend in the direction of increasing substrate-bound laminin concentrations, and that NGF gradients between 133 ng/mL per mm and 995 ng/mL per mm are needed to promote neurite outgrowth and guide growth cone extension in PC12 cells [91,92]. Likewise, collagen scaffolds cross-linked with laminin and loaded with CNTF resulted in enhanced axonal guidance, regeneration, and functional recovery in a rodent model of PNI [93]. In addition to NGF, factors such

as FGF are known to bind to heparin sulfate proteoglycans (HSPGs) of the ECM, which help protect it from proteolysis [94]. Additionally, vascular endothelial growth factor (VEGF), which plays a key role in angiogenesis, has been seen to increase nerve regeneration following PNI in rodents. VEGF is present in various isoforms, some of which remain diffusible, whereas other isoforms bind to HSPGs and form a chemoattractive gradient around VEGF-secreting cells that leads to vessel sprouting and ultimately promotes tissue growth [95]. The ability of ECM proteins to interact with cells and tissue makes them especially desirable as scaffolds for neural repair. In turn, adhesion to host structures and integration into the host microenvironment promotes implant function.

36.6.5 Incorporation of biomimetic and fibrillar contact-dependent cues

Not surprisingly, CAMs are highly upregulated under regenerative conditions [35]. Functionalized CAM biomimetics have also been shown to increase Schwann cell activity and myelination of regenerated axons [96,97]. Other structural guidance cues involve matrix proteins such as collagen, fibrin, and laminin, which may occur on cell surfaces but are most commonly associated with ECM (see Ref. [98] for recent review). The presence and density of these ECM ligands have been shown to be critical factors for axonal outgrowth in a number of model systems, including models of PNI and spinal cord injury [99–103]. Many ECM proteins have been incorporated into NGTs, such as collagen, laminin, fibronectin, hyaluronic acid (HA), and others, as discussed above.

Several additional techniques have been implemented to fabricate fibrillar scaffolds for neuroregeneration. A promising and relatively simple method of fabricating nanofibers is electrospinning. Here, an electric charge is used to draw liquid from a polymer in the form of a fiber with a micron-scale diameter [104]. Recently, electrospinning has been employed to develop nanofibers that recreate the structure, diameter, and tensile strength of neural ECM. For example, nanofibers exhibiting physical and mechanical properties similar to neural ECM were created by electrospinning poly(L-lactic acid)-co-poly(ε-caprolactone) (PLCL) to create nanofibers consisting of either blended laminin or PLCL-laminin core shell nanofibers, exhibiting physiologically relevant fiber diameter and tensile strength. Interestingly, an increase in Schwann cell proliferation on core shell nanofibers was seen. This was likely due to controlled release of the laminin from the core, which allowed for optimal levels of laminin for Schwann cell proliferation [105]. Laminin core-shell nanofibers may also prove beneficial if mixing bioactive molecules, such as NGF, is desired. Alignment has been seen to play an important role in promoting regeneration; aligned laminin-PCL blended fibers have been implemented as a repair strategy and resulted in increased nerve conduction speed at 6 weeks versus random nanofiber conduits [106]. Additionally, the use of Schwann cells seeded on electrospun HA-gelatin nanofibers for peripheral nerve regeneration was explored. Protein expression and F-actin alignment were increased when grown on nanofibers consisting of HA [107]. Interactions between regenerating axons with contact dependent cues, such as ECM proteins or other functionalized CAMs, may lead to increased nerve regeneration.

36.6.6 Optimization of physical properties

The mechanical and geometric properties of substrates are well known to effect cell outgrowth in 3D culture [108]. Likewise, substrate mechanical properties greatly influence neuronal behavior and axon outgrowth. Studies have shown that agarose stiffness and pore size differentially influenced the rate and degree of neurite extension of DRG neurons, with maximal neurite outgrowth occurring in low concentration (<1.00%) gels [46,100,109]. DRG neurite outgrowth has also been studied in collagen matrices of varying concentrations (and hence stiffness), finding that neurite extension was maximized in lower (0.6 mg/mL) rather than higher (2 mg/mL) concentration gels [103]; these studies underscore the sensitive nature of neurons/axons to matrix properties.

Additionally, the importance of geometric guidance cues, such as surface curvature, is increasingly being recognized [34,49,110,111]. For example, DRG axons in culture were shown to have enhanced longitudinal growth along microfibers with diameters of 35 μm or less–a diameter range similar to that of axon fascicles–due to the mechanics of minimizing process bending [34]. Additional surface features such as scratches, ridges, and grooves can aid in guiding neurite outgrowth. For instance, it was shown that growth cone branching was directly related to the number of potential paths at an intersection [112]. Similarly, the shape of the substrate affects neuron morphology and neuritogenesis. When neurons were cultured on varying micropatterned shapes, their cytoskeletons deformed to imitate the shape of the substrate, revealing that neuritogenesis was increased at the vertex of angles, especially at 60° angles [113]. Therefore, the physical/mechanical properties and the geometric presentation of surface/binding domains on a substrate greatly impact neurite behavior and can be used to manipulate neurite outgrowth.

36.7 Cell-based and tissue-engineered constructs

36.7.1 Cell-based nerve constructs

In addition to the loss of long-distance axonal tracts, PNI with large segmental defects also results in significant loss of support cells and tissue. In particular, it has been suggested that a primary reason for general failure of acellular nerve grafts (ANAs or NGTs) across long lesions (>3–5 cm) is that host Schwann cells run out of proliferative capacity and thus are unable to fully infiltrate and repopulate the graft region. Schwann cells also exhibit a neuroprotective effect in the PNS. Therefore, to promote regeneration, it may be necessary to replace lost Schwann cells directly and/or replenish them by implanting stem cells. As described previously, Schwann cells can act to maintain a proregenerative environment by directly interacting with the host milieu and modulating the concentration of proregenerative factors through feedback from the surrounding tissue. Thus, the inclusion of exogenous cells in scaffolds can prove to be extremely advantageous. ECM-based scaffolds or other vehicles are effective for cell transplantation due to their biocompatibility, cell protective, and cell adhesive properties. For example, when Schwann cells were added to an alginate matrix

containing fibronectin and subsequently transplanted into a rodent peripheral nerve lesion, conduits containing fibronectin displayed increased Schwann cell viability, leading to greater nerve regeneration. Recent studies have also found that transplantation of ECM-containing matrices increased Schwann cell attachment and viability. It should be noted that nerve regeneration was promoted due to the presence of Schwann cells, as well as the proregenerative effect of fibronectin [114]. Therefore, addition and/or preservation of Schwann cells should prove beneficial in achieving successful neural regeneration.

Because transplantation of mature glial cells, such as Schwann cells, may lead to an exacerbated host immune response, immature cells (e.g., Schwann cell progenitor cells) or stem cells are frequently chosen to seed scaffolds utilized for regeneration. Stem cell sources include embryonic stem cells, induced pluripotent stem cells, neural stem cells, and adult mesenchymal stem cells. Here, implanted stem cells may secrete proregenerative factors and/or differentiate into Schwann cells. Schwann cell-like bone marrow mesenchymal stems exhibit molecular and functional similarities to native Schwann cells and have been used as Schwann cell alternatives. These cells were embedded in Matrigel, inserted into commercially available fibers, and transplanted into the rat sciatic nerve. At 3 weeks post transplantation, robust nerve fiber regeneration was seen as well as myelination [115]. In another recent study, a laminin-coated chitosan scaffold seeded with bone marrow stem cells (BMSCs) was transplanted to repair a 10 mm peripheral nerve lesion. Although newly formed nerve cells covered the interior of the conduit even in the absence of BMSCs, the group treated with BMSC seeded conduit displayed increased regeneration as evidenced by increased nerve regrowth, muscle mass, functional recovery, and decreased neuronal death, inflammatory, and fibrotic responses [116].

36.7.2 Definition of living scaffolds

The field of regenerative medicine encompasses the use of biomaterials, cell replacement strategies, and tissue engineering to promote regeneration following injury or disease. As discussed above, biomaterials can provide 3D structure for host cell infiltration and organization and may also serve as a means for administration (e.g., controlled release) of soluble factors. Cell delivery strategies can replace lost cells in cases where endogenous cells are insufficient or unavailable (e.g., new Schwann cells). Tissue engineering combines aspects of both biomaterial and cell replacement techniques to create 3D constructs to facilitate regeneration of native tissue and/or to directly restore lost function based on permanent structural integration [117]. An emerging strategy in neural tissue engineering involves the development and application of "living scaffolds," which are defined as constructs with a controlled, often heterogeneous and anisotropic 3D cellular architecture and biomaterial composition. The cells impart the "living" component of the scaffold, and incorporated cell types may include primary, stem, differentiated, genetically engineered, autologous, allogeneic, or xenogeneic cells [117,118]. Living scaffolds may facilitate targeted neural cell migration and axonal pathfinding by mimicking key developmental mechanisms. As discussed previously, directed axon growth and cell migration along pathways formed

by other cells is a common tactic in nervous system development and is crucial to the proper formation of axonal connectivity and cellular localization. Growth and migration along living neural cells is driven by juxtacrine signaling involving the concurrent and often synergistic presentation of a panoply of cell-mediated haptotactic, chemotactic, and neurotrophic cues. Living scaffolds exploiting these cues possess considerable advantages over more traditional acellular biomaterial approaches due to the ability to *actively* drive and direct regeneration rather than simply being *permissive* substrates. Moreover, living scaffolds have the ability for constitutive and sustained interactions rather than transient, often short-lived influence on the host, as is the case with many acellular biomaterial-based approaches. Importantly, living scaffolds may act based on feedback and cross talk with regenerating cells/axons and thus are able to modulate their signaling based on the state and progression of the regenerative process. Biomaterials utilized within the scaffold often provide structure and produce an environment in which cells can adhere, migrate, differentiate, and signal to each other and to the host [119]. The biomaterial composition often governs the mechanical properties of the construct and resulting tissue [119]. A crucial property of a living scaffold is that it must possess a defined architecture, encompassing both the structural composition and the organization of the cells/processes. This architecture should be precisely engineered to match the structure and properties of the tissue it will integrate with or to provide directionality for infiltration and targeted regrowth of host cells. Biomaterials may be synthesized to promote such a desired cellular organization or to give directional dependence to mechanical properties, such as rigidity and elasticity [119]. Likewise, gradients of codelivered factors, such as growth factors and signaling molecules, may be used within living scaffolds to generate an anisotropic cytoarchitecture [33].

36.7.3 Examples of tissue-engineered living scaffolds in neuroregeneration

Several early in vitro studies paved the way for the later production and implementation of living scaffolds for neuroregeneration in vivo. In the last two decades, extensive progress has been made in the field of neural tissue engineering. The mechanisms of neural growth and axonal pathfinding discovered in the 1980s are now being fully utilized in tissue-engineered living scaffolds. Researchers have fabricated collagen constructs containing aligned rat Schwann cells for peripheral nerve regeneration, which they designated "engineered neural tissue." Aligned Schwann cells specifically recreate the bands of Bungner. Following transplantation of these fabricated tubes into a 15 mm gap rat sciatic nerve model, significantly more neural tissue was found in the experimental groups than control groups [120]. Other groups have engineered a collagen-based microstructured scaffold composed of longitudinally oriented and interconnected pores. In vitro studies showed that it was capable of inducing Schwann cell alignment and supporting longitudinal axon outgrowth. To test the efficacy of the construct in vivo, the porous collagen tube was seeded with Schwann cells and transplanted into a 20 mm sciatic nerve gap. It was found that the density of axons in the Schwann cell-seeded construct group was close to the density of axons in their autograft group, and that the two groups demonstrated comparable myelination [121].

An alternative approach to engineering scaffolds containing aligned glial cells is to create constructs containing long, aligned axonal tracts. These constructs are designed to utilize "axon-facilitated axon regeneration" to support host axon regrowth. This mechanism involves the growth of regenerating axons along preformed (i.e., tissue engineered) axonal pathways, mimicking axonal growth along "pioneer" axons during nervous system development. Our group has pioneered the technology to fabricate tissue-engineered nerve grafts (TENGs) that are composed of long aligned axonal tracts that can be utilized to repair long gap PNI. To generate TENGs, the well-established process of axonal "stretch growth" was implemented. This process mimics the developmental mechanism by which axons are extended in length due to tension as an organism grows from embryogenesis to adulthood [122,123]. This process involves plating two neuronal populations on either side of an interface, allowing axonal networks to form between them, and then slowly separating the populations in micron-size increments using custom mechanobioreactors. The modality of the constructs can be manipulated to better cater to the nerve being repaired. Historically, we have developed TENGs using pure sensory neuron "stretch-grown" axon tracts. These integrated axons respond to the forces by increasing in length and diameter, and this process also encourages fasciculation [122,123]. To date, stretch-grown axonal constructs have been generated at lengths up to 10 cm in 14–21 days, with even longer lengths likely attainable [122,123]. To test the efficacy of the TENGs in vivo, the stretch-grown axons were encapsulated in a collagenous matrix for stability and transferred into an NGT for transplantation into a 10–12 mm rat SNI model. TENG survival, maintenance of cytoarchitecture, and integration with host nerve tissue was observed. At 16 weeks post transplantation, the segments of neural tissue bridging the gap appeared grossly normal, with a significant density of myelinated host axons [124].

36.7.4 Advantages of tissue-engineered living scaffolds

A tissue-engineered living scaffold with the highest capacity for regeneration will possess all of the aforementioned factors, including chemotactic, haptotactic, and mechanical cues, which will work synergistically to promote targeted axonal guidance and/or cellular infiltration. Although creating 3D living scaffolds represents a tremendously complex endeavor, significant progress has been made in the past decade. These living scaffolds show great promise for neuroregeneration and hold significant advantages over competing regenerative therapies. Living cells possess the ability to secrete thousands of neurotrophic factors and control CAM expression. They may actively respond to their environment and modulate proregenerative cues such that they remain optimal for axon guidance and sprouting, myelination, and the restoration of complex 3D tissue structures. In contrast to living scaffolds, most current axon guidance conduits or acellular scaffolds are fabricated from synthetic or naturally occurring biomaterials. As discussed above, these constructs are sometimes loaded or coated with soluble factors to promote neural regeneration [33,95,117]. Such therapies are limited in recreating and maintaining the optimal concentrations of soluble factors and presentation of structural cues for regeneration; they weakly simulate the conditions that exist during embryonic development when neurogenesis and axogenesis first occur.

At present, nonliving scaffolds can only deliver a relatively small number of factors involved in regeneration, and, although controlled release of soluble factors is a common objective [95,117,125], acellular scaffolds are not currently capable of modulating secreted factors based on the progression and state of the regenerative process. As such, the mechanisms and efficacy of numerous acellular constructs have not successfully translated to in vivo environments despite being "optimized" in vitro, suggesting that endogenous processes and signals may override the regenerative effects of many contemporary acellular biomaterials.

36.8 Conclusion

Long-distance peripheral nerve regeneration remains a challenge as current clinical strategies fail to recreate the microenvironment necessary for long-distance axonal growth. The SNI model is the most prevalent tool to study and develop novel repair strategies for PNI, as it is easily accessible and clinically relevant. The ideal proregenerative environment is one that closely mimics in vivo developmental conditions and provides robust feedback to modulate regeneration. Therefore, novel nerve repair strategies should be inspired from developmental axon pathfinding and therefore augment natural neuroregeneration process, as both topographical and chemical cues are necessary to facilitate axon outgrowth. As such, "living", cell-based scaffolds are able to effectively promote regeneration by serving as an *active* substrate for axon pathfinding by providing the necessary cues for regenerating axons. However, there are several significant challenges to the development and translation of living biological scaffolds, including advancing tissue engineering techniques for the creation of living cellular constructs in a defined 3D architecture, establishing transplantation strategies to ensure preservation of construct vitality and architecture, and devising strategies for immunological tolerance at both acute and chronic time frames. As these challenges are overcome, living scaffolds have the potential to transform the field of neuroregenerative medicine by driving the reestablishment of complex neural structures and axonal connections, ultimately facilitating functional recovery following a range of currently untreatable traumatic nerve injuries.

References

[1] H. Gray, H. Gray, Anatomy of the Human Body, Lea & Febiger, Philadelphia, 1918, Online edition: Bartleby.com. 2000.
[2] E. Wang, et al., Optimal timing for repair of peripheral nerve injuries, J Trauma Acute Care Surg 83 (5) (2017) 875–881.
[3] L.R. Robinson, Traumatic injury to peripheral nerves, Muscle Nerve 23 (6) (2000) 863–873.
[4] B.J. Pfister, et al., Biomedical engineering strategies for peripheral nerve repair: surgical applications, state of the art, and future challenges, Crit Rev Biomed Eng 39 (2) (2011) 81–124.

[5] Z.S. Ali, et al., Utility of delayed surgical repair of neonatal brachial plexus palsy, J Neurosurg Pediatr 13 (4) (2014) 462–470.
[6] Z.S. Ali, et al., Upper brachial plexus injury in adults: comparative effectiveness of different repair techniques, J Neurosurg 122 (1) (2015) 195–201.
[7] E.L. Zager, For this peripheral nerve lesion, it is best to avoid the knife, World Neurosurg 82 (3–4) (2014) 333–334.
[8] S.K. Lee, S.W. Wolfe, Peripheral nerve injury and repair, J Am Acad Orthop Surg 8 (4) (2000) 243–252.
[9] A.C. Ruijs, et al., Median and ulnar nerve injuries: a meta-analysis of predictors of motor and sensory recovery after modern microsurgical nerve repair, Plast Reconstr Surg 116 (2) (2005) 484–494 discussion 495-6.
[10] F. Eser, et al., Etiological factors of traumatic peripheral nerve injuries, Neurol India 57 (4) (2009) 434–437.
[11] R. Birch, et al., Experience with the free vascularized ulnar nerve graft in repair of supraclavicular lesions of the brachial plexus, Clin Orthop Relat Res (237) (1988) 96–104.
[12] K.L. Colen, M. Choi, D.T. Chiu, Nerve grafts and conduits, Plast Reconstr Surg 124 (6 Suppl. l) (2009) e386–e394.
[13] G.I. Taylor, F.J. Ham, The free vascularized nerve graft. A further experimental and clinical application of microvascular techniques, Plast Reconstr Surg 57 (4) (1976) 413–426.
[14] D. Arslantunali, et al., Peripheral nerve conduits: technology update, Med Devices (Auckl) 7 (2014) 405–424.
[15] B. Battiston, et al., Chapter 11: tissue engineering of peripheral nerves, Int Rev Neurobiol 87 (2009) 227–249.
[16] T. Scholz, et al., Peripheral nerve injuries: an international survey of current treatments and future perspectives, J Reconstr Microsurg 25 (6) (2009) 339–344.
[17] K. Bhatheja, J. Field, Schwann cells: origins and role in axonal maintenance and regeneration, Int J Biochem Cell Biol 38 (12) (2006) 1995–1999.
[18] D.H. Kim, et al., Kline and Hudson's Nerve Injuries: Operative Results for Major Nerve Injuries, Entrapments and Tumors, Elsevier Health Sciences, 2008.
[19] H. Schmalbruch, Fiber composition of the rat sciatic nerve, Anat Rec 215 (1) (1986) 71–81.
[20] S. Sunderland, Nerves and Nerve in Juries, E& S Livingstone Ltd, Edinburgh, 1968.
[21] J. Raper, C. Mason, Cellular strategies of axonal pathfinding, Cold Spring Harb Perspect Biol 2 (9) (2010) a001933.
[22] J.A. Raper, M. Bastiani, C.S. Goodman, Pathfinding by neuronal growth cones in grasshopper embryos. II. Selective fasciculation onto specific axonal pathways, J Neurosci 3 (1) (1983) 31–41.
[23] K.J. Sepp, J. Schulte, V.J. Auld, Peripheral glia direct axon guidance across the CNS/PNS transition zone, Dev Biol 238 (1) (2001) 47–63.
[24] J.R. Jacobs, C.S. Goodman, Embryonic development of axon pathways in the Drosophila CNS. I. A glial scaffold appears before the first growth cones, J Neurosci 9 (7) (1989) 2402–2411.
[25] D. Bentley, M. Caudy, Pioneer axons lose directed growth after selective killing of guidepost cells, Nature 304 (5921) (1983) 62–65.
[26] T.A. Evans, G.J. Bashaw, Axon guidance at the midline: of mice and flies, Curr Opin Neurobiol 20 (1) (2010) 79–85.
[27] R.K. Ho, C.S. Goodman, Peripheral pathways are pioneered by an array of central and peripheral neurones in grasshopper embryos, Nature 297 (5865) (1982) 404–406.
[28] L. Wang, T. Marquardt, What axons tell each other: axon-axon signaling in nerve and circuit assembly, Curr Opin Neurobiol 23 (6) (2013) 974–982.

[29] G. Xu, et al., Optic nerve regeneration in polyglycolic acid-chitosan conduits coated with recombinant L1-Fc, Neuroreport 15 (14) (2004) 2167–2172.
[30] Q. Yan, Y. Yin, B. Li, Use new PLGL-RGD-NGF nerve conduits for promoting peripheral nerve regeneration, Biomed Eng Online 11 (2012) 36.
[31] E.G. Fine, et al., GDNF and NGF released by synthetic guidance channels support sciatic nerve regeneration across a long gap, Eur J Neurosci 15 (4) (2002) 589–601.
[32] P.J. Apel, et al., Effect of locally delivered IGF-1 on nerve regeneration during aging: an experimental study in rats, Muscle Nerve 41 (3) (2010) 335–341.
[33] S. Madduri, et al., Effect of controlled co-delivery of synergistic neurotrophic factors on early nerve regeneration in rats, Biomaterials 31 (32) (2010) 8402–8409.
[34] R.M. Smeal, et al., Substrate curvature influences the direction of nerve outgrowth, Ann Biomed Eng 33 (3) (2005) 376–382.
[35] A.E. Wiencken-Barger, et al., The role of L1 in axon pathfinding and fasciculation, Cerebr Cortex 14 (2) (2004) 121–131.
[36] P. Shi, K. Shen, L.C. Kam, Local presentation of L1 and N-cadherin in multicomponent, microscale patterns differentially direct neuron function in vitro, Dev Neurobiol 67 (13) (2007) 1765–1776.
[37] J.K. Chilton, Molecular mechanisms of axon guidance, Dev Biol 292 (1) (2006) 13–24.
[38] R.J. Giger, E.R. Hollis 2nd, M.H. Tuszynski, Guidance molecules in axon regeneration, Cold Spring Harb Perspect Biol 2 (7) (2010) a001867.
[39] G. Loers, M. Schachner, Recognition molecules and neural repair, J Neurochem 101 (4) (2007) 865–882.
[40] C. Plachez, L.J. Richards, Mechanisms of axon guidance in the developing nervous system, Curr Top Dev Biol 69 (2005) 267–346.
[41] E.Y. Van Battum, S. Brignani, R.J. Pasterkamp, Axon guidance proteins in neurological disorders, Lancet Neurol 14 (5) (2015) 532–546.
[42] D. Arvanitis, A. Davy, Eph/ephrin signaling: networks, Genes Dev 22 (4) (2008) 416–429.
[43] R. Klein, A. Kania, Ephrin signalling in the developing nervous system, Curr Opin Neurobiol 27 (2014) 16–24.
[44] J.G. Boyd, T. Gordon, Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury, Mol Neurobiol 27 (3) (2003) 277–324.
[45] P.C. Georges, et al., Matrices with compliance comparable to that of brain tissue select neuronal over glial growth in mixed cortical cultures, Biophys J 90 (8) (2006) 3012–3018.
[46] A.P. Balgude, et al., Agarose gel stiffness determines rate of DRG neurite extension in 3D cultures, Biomaterials 22 (10) (2001) 1077–1084.
[47] L.A. Flanagan, et al., Neurite branching on deformable substrates, Neuroreport 13 (18) (2002) 2411–2415.
[48] A. Ayali, The function of mechanical tension in neuronal and network development, Integr Biol (Camb) 2 (4) (2010) 178–182.
[49] R.M. Smeal, P.A. Tresco, The influence of substrate curvature on neurite outgrowth is cell type dependent, Exp Neurol 213 (2) (2008) 281–292.
[50] F. Johansson, et al., Axonal outgrowth on nano-imprinted patterns, Biomaterials 27 (8) (2006) 1251–1258.
[51] B.J. Pfister, et al., Stretch-grown axons retain the ability to transmit active electrical signals, FEBS Lett 580 (14) (2006) 3525–3531.
[52] J.W. Fawcett, R.J. Keynes, Peripheral nerve regeneration, Annu Rev Neurosci 13 (1990) 43–60.

[53] G.R. Evans, Peripheral nerve injury: a review and approach to tissue engineered constructs, Anat Rec 263 (4) (2001) 396–404.
[54] S.E. Mackinnon, New directions in peripheral nerve surgery, Ann Plast Surg 22 (3) (1989) 257–273.
[55] V.R. Hentz, et al., The nerve gap dilemma: a comparison of nerves repaired end to end under tension with nerve grafts in a primate model, J Hand Surg Am 18 (3) (1993) 417–425.
[56] H.M. Kaplan, P. Mishra, J. Kohn, The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans, J Mater Sci Mater Med 26 (8) (2015) 226.
[57] C.M. Nichols, et al., Effects of motor versus sensory nerve grafts on peripheral nerve regeneration, Exp Neurol 190 (2) (2004) 347–355.
[58] S. Geuna, et al., Chapter 3: histology of the peripheral nerve and changes occurring during nerve regeneration, Int Rev Neurobiol 87 (2009) 27–46.
[59] S.W. Kemp, P.S. Cederna, R. Midha, Comparative outcome measures in peripheral regeneration studies, Exp Neurol 287 (Pt 3) (2017) 348–357.
[60] C.A. Munro, et al., Lack of association between outcome measures of nerve regeneration, Muscle Nerve 21 (8) (1998) 1095–1097.
[61] A. Rupp, et al., Strain differences in the branching of the sciatic nerve in rats, Anat Histol Embryol 36 (3) (2007) 202–208.
[62] M.C. Dodla, R.V. Bellamkonda, Differences between the effect of anisotropic and isotropic laminin and nerve growth factor presenting scaffolds on nerve regeneration across long peripheral nerve gaps, Biomaterials 29 (1) (2008) 33–46.
[63] L. de Medinaceli, W.J. Freed, R.J. Wyatt, An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks, Exp Neurol 77 (3) (1982) 634–643.
[64] A.S. Varejão, et al., Functional evaluation of peripheral nerve regeneration in the rat: walking track analysis, J Neurosci Methods 108 (1) (2001) 1–9.
[65] A.S. Varejão, et al., Methods for the experimental functional assessment of rat sciatic nerve regeneration, Neurol Res 26 (2) (2004) 186–194.
[66] A. Bozkurt, et al., Catwalk gait analysis in assessment of functional recovery after sciatic nerve injury, J Neurosci Methods 173 (1) (2008) 91–98.
[67] L.M. Costa, et al., Methods and protocols in peripheral nerve regeneration experimental research: part IV—kinematic gait analysis to quantify peripheral nerve regeneration in the rat, Int Rev Neurobiol 87 (2009) 127–139.
[68] X. Navarro, E. Udina, Methods and protocols in peripheral nerve regeneration experimental research: Part III—electrophysiological evaluation, Int Rev Neurobiol 87 (2009) 105–126.
[69] T.H. Nijhuis, et al., Natural conduits for bridging a 15-mm nerve defect: comparison of the vein supported by muscle and bone marrow stromal cells with a nerve autograft, J Plast Reconstr Aesthetic Surg 66 (2) (2013) 251–259.
[70] X. Chen, et al., Ultrasound imaging of chitosan nerve conduits that bridge sciatic nerve defects in rats, Neural Regen Res 9 (14) (2014) 1386.
[71] B. Behr, et al., Magnetic resonance imaging monitoring of peripheral nerve regeneration following neurotmesis at 4.7 Tesla, Plast Reconstr Surg 123 (6) (2009) 1778–1788.
[72] C.-D. Liao, et al., Peripheral nerve repair: monitoring by using gadofluorine M–enhanced MR imaging with chitosan nerve conduits with cultured mesenchymal stem cells in rat model of neurotmesis, Radiology 262 (1) (2012) 161–171.
[73] T. Yamasaki, et al., In vivo evaluation of rabbit sciatic nerve regeneration with diffusion tensor imaging (DTI): correlations with histology and behavior, Magn Reson Imag 33 (1) (2015) 95–101.

[74] P. Konofaos, J.P. Ver Halen, Nerve repair by means of tubulization: past, present, future, J Reconstr Microsurg 29 (3) (2013) 149–164.
[75] G.C. de Ruiter, et al., Designing ideal conduits for peripheral nerve repair, Neurosurg Focus 26 (2) (2009) E5.
[76] A.M. Moore, et al., Acellular nerve allografts in peripheral nerve regeneration: a comparative study, Muscle Nerve 44 (2) (2011) 221–234.
[77] T.M. Myckatyn, S.E. Mackinnon, A review of research endeavors to optimize peripheral nerve reconstruction, Neurol Res 26 (2) (2004) 124–138.
[78] P. Tang, et al., Histologic and functional outcomes of nerve defects treated with acellular allograft versus cabled autograft in a rat model, Microsurgery 33 (6) (2013) 460–467.
[79] E.L. Whitlock, et al., Processed allografts and type I collagen conduits for repair of peripheral nerve gaps, Muscle Nerve 39 (6) (2009) 787–799.
[80] P.J. Johnson, et al., Nerve endoneurial microstructure facilitates uniform distribution of regenerative fibers: a post hoc comparison of midgraft nerve fiber densities, J Reconstr Microsurg 27 (2) (2011) 83–90.
[81] Z.A. Accioli-De-Vaconcellos, L. Kassar-Duchossoy, J.C. Mira, Long term evaluation of experimental median nerve repair by frozen and fresh nerve autografts, allografts and allografts repopulated by autologous Schwann cells, Restor Neurol Neurosci 15 (1) (1999) 17–24.
[82] Y. Zhang, et al., Combining acellular nerve allografts with brain-derived neurotrophic factor transfected bone marrow mesenchymal stem cells restores sciatic nerve injury better than either intervention alone, Neural Regen Res 9 (20) (2014) 1814–1819.
[83] Z. Zhu, et al., Ginkgo biloba extract (EGb 761) promotes peripheral nerve regeneration and neovascularization after acellular nerve allografts in a rat model, Cell Mol Neurobiol 35 (2) (2015) 273–282.
[84] Y.S. Chen, et al., Peripheral nerve regeneration using silicone rubber chambers filled with collagen, laminin and fibronectin, Biomaterials 21 (15) (2000) 1541–1547.
[85] X.J. Tong, et al., Sciatic nerve regeneration navigated by laminin-fibronectin double coated biodegradable collagen grafts in rats, Brain Res 663 (1) (1994) 155–162.
[86] W. Yu, et al., Sciatic nerve regeneration in rats by a promising electrospun collagen/poly(epsilon-caprolactone) nerve conduit with tailored degradation rate, BMC Neurosci 12 (2011) 68.
[87] S. Nichterwitz, et al., Bioengineered glial strands for nerve regeneration, Neurosci Lett 484 (2) (2010) 118–122.
[88] E. Verdu, et al., Alignment of collagen and laminin-containing gels improve nerve regeneration within silicone tubes, Restor Neurol Neurosci 20 (5) (2002) 169–179.
[89] S.H. Hsu, C.H. Su, I.M. Chiu, A novel approach to align adult neural stem cells on micropatterned conduits for peripheral nerve regeneration: a feasibility study, Artif Organs 33 (1) (2009) 26–35.
[90] A. Faroni, et al., Peripheral nerve regeneration: experimental strategies and future perspectives, Adv Drug Deliv Rev 82–83 (2015) 160–167.
[91] X. Cao, M.S. Shoichet, Defining the concentration gradient of nerve growth factor for guided neurite outgrowth, Neuroscience 103 (3) (2001) 831–840.
[92] S.K. Dertinger, et al., Gradients of substrate-bound laminin orient axonal specification of neurons, Proc Natl Acad Sci USA 99 (20) (2002) 12542–12547.
[93] J. Cao, et al., The use of laminin modified linear ordered collagen scaffolds loaded with laminin-binding ciliary neurotrophic factor for sciatic nerve regeneration in rats, Biomaterials 32 (16) (2011) 3939–3948.
[94] M.L. Reynolds, C.J. Woolf, Reciprocal Schwann cell-axon interactions, Curr Opin Neurobiol 3 (5) (1993) 683–693.

[95] J.M. Rosenstein, J.M. Krum, C. Ruhrberg, VEGF in the nervous system, Organogenesis 6 (2) (2010) 107–114.
[96] Y. Haile, et al., Culturing of glial and neuronal cells on polysialic acid, Biomaterials 28 (6) (2007) 1163–1173.
[97] A. Mehanna, et al., Polysialic acid glycomimetics promote myelination and functional recovery after peripheral nerve injury in mice, Brain 132 (Pt 6) (2009) 1449–1462.
[98] J.P. Myers, M. Santiago-Medina, T.M. Gomez, Regulation of axonal outgrowth and pathfinding by integrin-ECM interactions, Dev Neurobiol 71 (11) (2011) 901–923.
[99] R. Bellamkonda, J.P. Ranieri, P. Aebischer, Laminin oligopeptide derivatized agarose gels allow three-dimensional neurite extension in vitro, J Neurosci Res 41 (4) (1995) 501–509.
[100] R. Bellamkonda, et al., Hydrogel-based three-dimensional matrix for neural cells, J Biomed Mater Res 29 (5) (1995) 663–671.
[101] C. Krewson, et al., Cell-aggregation and neurite growth in gels of extracellular-matrix molecules, Biotechnol Bioeng 43 (7) (1994) 555–562.
[102] J.C. Schense, J.A. Hubbell, Three-dimensional migration of neurites is mediated by adhesion site density and affinity, J Biol Chem 275 (10) (2000) 6813–6818.
[103] R.K. Willits, S.L. Skornia, Effect of collagen gel stiffness on neurite extension, J Biomater Sci Polym Ed 15 (12) (2004) 1521–1531.
[104] A. Ziabacki, Fundamentals of Fiber Formation, Wiley and Sons, New York, 1976.
[105] E. Kijeńska, et al., Interaction of Schwann cells with laminin encapsulated PLCL core–shell nanofibers for nerve tissue engineering, Eur Polym J 50 (2014) 30–38.
[106] R.A. Neal, et al., Alignment and composition of laminin-polycaprolactone nanofiber blends enhance peripheral nerve regeneration, J Biomed Mater Res 100 (2) (2012) 406–423.
[107] H.-M. Liou, L.-R., Rau, C.-C. Huang, M.-R. Lu, F.-Y. Hsu, Electrospun hualuronan-gelatin nanofibrous matrix for nerve tissue engineering, J Nanomater 2013 (2013) 6.
[108] D.E. Discher, P. Janmey, Y.L. Wang, Tissue cells feel and respond to the stiffness of their substrate, Science 310 (5751) (2005) 1139–1143.
[109] G.P. Dillon, et al., The influence of physical structure and charge on neurite extension in a 3D hydrogel scaffold, J Biomater Sci Polym Ed 9 (10) (1998) 1049–1069.
[110] D.K. Cullen, et al., Developing a tissue-engineered neural-electrical relay using encapsulated neuronal constructs on conducting polymer fibers, J Neural Eng 5 (4) (2008) 374–384.
[111] X. Wen, P.A. Tresco, Effect of filament diameter and extracellular matrix molecule precoating on neurite outgrowth and Schwann cell behavior on multifilament entubulation bridging device in vitro, J Biomed Mater Res 76 (3) (2006) 626–637.
[112] G.S. Withers, et al., Effects of substrate geometry on growth cone behavior and axon branching, J Neurobiol 66 (11) (2006) 1183–1194.
[113] M.J. Jang, Y. Nam, Geometric effect of cell adhesive polygonal micropatterns on neuritogenesis and axon guidance, J Neural Eng 9 (4) (2012) 046019.
[114] A. Mosahebi, M. Wiberg, G. Terenghi, Addition of fibronectin to alginate matrix improves peripheral nerve regeneration in tissue-engineered conduits, Tissue Eng 9 (2) (2003) 209–218.
[115] M. Dezawa, et al., Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells, Eur J Neurosci 14 (11) (2001) 1771–1776.
[116] S.H. Hsu, et al., New nerve regeneration strategy combining laminin-coated chitosan conduits and stem cell therapy, Acta Biomater 9 (5) (2013) 6606–6615.
[117] D. Eberli, A. Atala, Tissue engineering using adult stem cells, Methods Enzymol 420 (2006) 287–302.

[118] J.A. Korecka, J. Verhaagen, E.M. Hol, Cell-replacement and gene-therapy strategies for Parkinson's and Alzheimer's disease, Regen Med 2 (4) (2007) 425–446.
[119] B.P. Chan, K.W. Leong, Scaffolding in tissue engineering: general approaches and tissue-specific considerations, Eur Spine J 17 (Suppl. 4) (2008) 467–479.
[120] M. Georgiou, et al., Engineered neural tissue for peripheral nerve repair, Biomaterials 34 (30) (2013) 7335–7343.
[121] A. Bozkurt, et al., The role of microstructured and interconnected pore channels in a collagen-based nerve guide on axonal regeneration in peripheral nerves, Biomaterials 33 (5) (2012) 1363–1375.
[122] B.J. Pfister, et al., Extreme stretch growth of integrated axons, J Neurosci 24 (36) (2004) 7978–7983.
[123] B.J. Pfister, et al., Development of transplantable nervous tissue constructs comprised of stretch-grown axons, J Neurosci Methods 153 (1) (2006) 95–103.
[124] J.H. Huang, et al., Long-term survival and integration of transplanted engineered nervous tissue constructs promotes peripheral nerve regeneration, Tissue Eng 15 (7) (2009) 1677–1685.
[125] R.G. Thorne, W.H. Frey 2nd, Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations, Clin Pharmacokinet 40 (12) (2001) 907–946.

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