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Two pole air gap electrospinning: Fabrication of highly aligned, three-dimensional scaffolds for nerve reconstruction

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ABSTRACT

We describe the structural and functional properties of three-dimensional (3D) nerve guides fabricated from poly-ε-caprolactone (PCL) using the air gap electrospinning process. This process makes it possible to deposit nano-to-micron diameter fibers into linear bundles that are aligned in parallel with the long axis of a cylindrical construct. By varying starting electrospinning conditions it is possible to modulate scaffold material properties and void space volume. The architecture of these constructs provides thousands of potential channels to direct axon growth. In cell culture functional assays, scaffolds composed of individual PCL fibers ranging from 400 to 1500 nm supported the penetration and growth of axons from rat dorsal root ganglion. To test the efficacy of our guide design we reconstructed 10 mm lesions in the rodent sciatic nerve with scaffolds that had fibers 1 μm in average diameter and void volumes >90%. Seven weeks post implantation, microscopic examination of the regenerating tissue revealed dense, parallel arrays of myelinated and non-myelinated axons. Functional blood vessels were scattered throughout the implant. We speculate that end organ targeting might be improved in nerve injuries if axons can be directed to regenerate along specific tissue planes by a guide composed of 3D fiber arrays.

1. Introduction

After an injury, peripheral nerves can undergo an astounding degree of regeneration. When a nerve is severed, all signals distal to the injury site are immediately lost. Over time, downstream axons undergo Wallerian degeneration [1]. The surviving nerves of the proximal segment subsequently begin to undergo regeneration in response to soluble factors, many of which are produced by Schwann cells [2,3]. If the precipitating injury cleanly severs the nerve, treatment may be confined to a surgery that is designed to re-establish the continuity between the truncated stumps of the damaged nerve. In this surgery the proximal and the distal aspects of the perineural sheath are sutured together to form an end-to-end anastomosis. In more extreme injuries where a long segment of the nerve is crushed or completely lost, treatment is greatly complicated. Under these conditions a conduit, or nerve guide, is used to bridge the gap and direct the regenerating axons to grow towards the distal stump.

Nerve guides have a relatively long clinical history; these tubular constructs are designed to direct the natural processes that lead to regeneration [4,5]. A variety of natural and bioengineered materials have been used in this type of application, with mixed success [6]. Early synthetic guides consisted of a simple, hollow tube that provided little more than a protected environment [7]. Next generation nerve guides have been fabricated to contain signal molecules [8] and/or structural features [9] that are intended to provide guidance cues to the regenerating axons. Functional recovery with these constructs can be quite extensive, as long as the nerve guide is used to bridge a gap of less than about 10 mm in length. Once the injury gap exceeds this threshold, the regeneration process will be compromised to varying degrees. Typically, in these injuries only a limited number of axons will actually traverse the wound bed and the efficiency of targeting to the distal tissues is poor, resulting in limited functional recovery. Further exacerbating these complications, a series of irreversible degenerative changes begins to evolve in the distal tissues. As these degenerative changes become entrenched, the prospects of meaningful functional recovery are greatly diminished, even if a large number of axons are efficiently targeted to these sites.

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Several published studies have demonstrated that aligned arrays of electrospun fibers can provide the guidance cues necessary to induce axons and glial cells to express a highly polarized phenotype [10–13]. Despite these preliminary and encouraging results, it is difficult to fabricate a clinically relevant nerve guide using the conventional electrospinning process, which uses a rapidly rotating target mandrel to produce aligned fibers. Conventional electrospinning systems are very effective at producing flat, two-dimensional (2D) sheets with highly anisotropic fibers [14–16]. These constructs are easily amenable to experimentation in vitro; however, a 2D sheet is less acceptable, or adaptable, for use in vivo. Despite these limitations, the efficacy of using the conventional electrospinning process to fabricate hollow, cylindrical nerve guides has been explored to some extent. In these experiments the electrospun fibers have been deposited onto a round, rotating mandrel. While the fibers of this type of construct can be induced to exhibit a considerable degree of alignment when produced under these conditions, the fibers, unfortunately, are deposited onto the target mandrel in a circumferential orientation (i.e. the axis of alignment is ∼90° off with respect to the long axis of the hollow tube). For obvious reasons this architectural pattern does not lend itself well to providing guidance cues to regenerating axons. However, the nano-to-micron diameter fibers that form the wall of this type of construct do represent an effective barrier that reduces the risk of inflammatory cells penetrating into the lumen of the guide. Blocking the infiltration of these cells into the lumen of a nerve guide is critical to the regeneration process [17,18].

In our view, a more ideal nerve guide configuration closely mimics the structure of the autologous nerve graft. This idealized guide would display a cylindrical shape and be composed of dense three-dimensional (3D) arrays of highly aligned electrospun fibers that were oriented in parallel with the long axis of the construct. The gaps and elongated spaces that will exist between the stacked fiber arrays would literally provide thousands of channels for directed axonal growth. This type of configuration can be approximated by rolling a 2D sheet of aligned fibers into a cylinder. Unfortunately, this will result in a seam in the construct that could provide an avenue for the infiltration and penetration of inflammatory and interstitial cells into the construct. The seam could also represent a nexus for mechanical failure. Air gap electrospinning makes it possible to circumvent these limitations and produce cylindrical, seamless, and truly 3D constructs composed of aligned arrays of electrospun fibers that are oriented in parallel with the long axis of the cylindrical construct.

In this study, we characterize the air gap electrospinning process and examine how this fabrication technique can be exploited to produce nerve guides that facilitate the regeneration of peripheral nerve fibers. We chose poly-ε-caprolactone (PCL) as the polymer for the manufacture of the nerve guide owing to its slow rate of degradation [19]; thus it can act as a guide for the new axons throughout the nerve regeneration process. PCL was electrospun at varying concentrations for in vitro testing to determine the most appropriate air gap electrospinning variables for the manufacture of constructs suitable for directing the axons in peripheral nerve injuries.

2. Methods

2.1. Electrospinning

PCL (Sigma: PCL 65,000 MW) was dissolved in trifluoroethanol (TFE) at various concentrations, including 50, 75, 100, 125, 150, 175, 200, 225, 250 and 275 mg ml^{-1}. Solutions were loaded into a 10 ml syringe that was capped with an 18 gauge blunt-tipped needle. Conductive, circular washers of varying diameters were placed over the blunt-tipped needle; several different electrospinning configurations were tested at each concentration of PCL. The air gap electrospinning system used in this study consists of two vertical piers grounded to a common voltage, typically set to −4.0 to −16.0 kV (Fig. 1, point A). From each vertical pier an additional set of horizontal piers project inwards at 90° with respect to the upright piers (Fig. 1, point B). A gap (which can be adjusted from about 1 to 6 inches) separates the terminal ends of these projecting piers. Electrospinning solutions were charged to +22 kV and directed into the gap separating the grounded horizontal piers. Polymer solutions were metered into the air gap system using a syringe driver with rates of delivery varying from 2 to 20 ml h^{-1} (see Table 1 for specific conditions). The distance between the solution reservoir and ground target array was varied from 10 cm to 30 cm. Once the charged electrospinning jet forms in this type of spinning, the polymer stream reaches the target and is laid out in a series of loops that pass back and forth between the terminal portions of the grounded piers, resulting in the formation of a bundle of parallel fiber arrays (Fig. 1, point C). Conditions for electrospinning at each PCL concentration were optimized to maximize fiber formation and collection onto the mandrel. We note that the electrospinning conditions disclosed in Table 1 are optimized to our specific laboratory environment (e.g., 68°F with ∼40% humidity) and electrospinning cabinet. Adjustments may be necessary to account for varying ambient conditions and different polymers.

2.2. Routine scanning electron microscopy (SEM)

A Zeiss EVO XVP scanning electron microscope equipped with digital acquisition was used for image capture. Electrospun constructs were removed from the target and cut into three sections of equal length. Each of the three segments was mounted onto a scanning electron microscope stud and sputter-coated. Average fiber diameter was determined from $N = 3–5$ SEM images captured at magnifications ranging from 450 to 5000× from each of the three sections using the NIH ImageTool software [17].

2.3. Fiber alignment analysis

Using the digital SEM images captured for fiber diameter analysis, the relative degree of fiber alignment was measured in each guide segment using the NIH ImageJ 2D fast Fourier transform (2D FFT) function [14,20]. By using the 2D FFT approach an align-

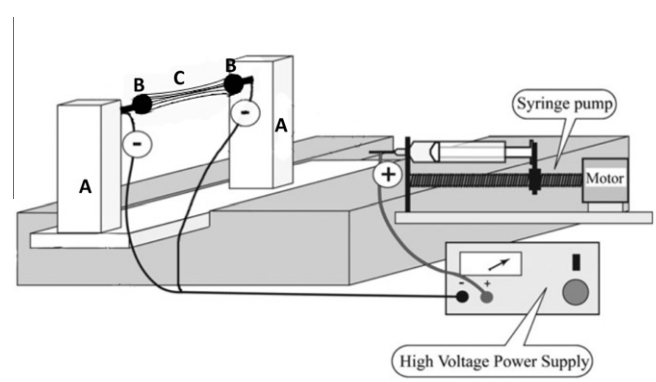

Fig. 1. Schematic of the ground target used in a two pole air gap electrospinning system. (A) Vertical, hollow piers transmit ground wires to horizontal piers (B) that project inward towards one another. (C) Fibers accumulate across the gap that separates the two horizontal piers. The positions of the syringe and electrospinning source are relatively arbitrary and largely dependent upon the cabinet used for electroprocessing. For this study we positioned the electrospinning source parallel to the base of the ground device and at a height that corresponded to the height of the grounded horizontal piers (B).