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Thermally drawn fibers as nerve guidance scaffolds

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ABSTRACT

Synthetic neural scaffolds hold promise to eventually replace nerve autografts for tissue repair following peripheral nerve injury. Despite substantial evidence for the influence of scaffold geometry and dimensions on the rate of axonal growth, systematic evaluation of these parameters remains a challenge due to limitations in materials processing. We have employed fiber drawing to engineer a wide spectrum of polymer-based neural scaffolds with varied geometries and core sizes. Using isolated whole dorsal root ganglia as an *in vitro* model system we have identified key features enhancing nerve growth within these fiber scaffolds. Our approach enabled straightforward integration of microscopic topography at the scale of nerve fascicles within the scaffold cores, which led to accelerated Schwann cell migration, as well as neurite growth and alignment. Our findings indicate that fiber drawing provides a scalable and versatile strategy for producing nerve guidance channels capable of controlling direction and accelerating the rate of axonal growth.

1. Introduction

Injuries to the peripheral nervous system (PNS) affect a broad population globally and often result in life-long disabilities in 60% of the patients due to the limited regenerative ability of neural tissue [1]. Following PNS injury, regenerating axons from the proximal nerve stump have to span the injury site and reconnect with the distal targets. While spontaneous recovery can occur for small-gap injuries (less than 2 cm), regeneration across larger injuries is impeded by a combination of factors including immune response, scarring, poor support cell repopulation, and neuronal death [2,3]. The common PNS surgical intervention for small-gap injuries, fascicular neurorrhaphy that sutures the ends of the proximal and distal nerve stumps together, adequately restores function only in ~50% of patients [4]. Autografting of the donor tissue is commonly used for injuries greater than 2 cm [5]. While ubiquitous in clinic, this method is limited by the availability of donor tissue, and poses a risk of secondary co-morbidity and neuroma formation [6,7]. For complete nerve transections with gap distances greater than 4 cm, functional recovery becomes highly unlikely even with surgical intervention [8].

Nerve guidance scaffolds promoting axonal growth may in future provide therapeutic alternatives to autografts. While a variety of synthetic and biopolymers, such as collagen, polycaprolactone, polyglycolic acid, poly-DL-lactide-co-caprolactone (PLCL), and polyvinyl alcohol (PVA) [9], have been explored as scaffold materials, geometry of these devices remains largely limited to simple cylindrical lumens with millimeter dimensions [10–13]. Since individual fascicle dimensions are on the order of microns, which is ~1000 times smaller than typical scaffolds, the role of the channel size on Schwann cell migration and axonal growth remains poorly understood especially for channels smaller than 200 μm. To date, limited work on systematically investigating the effects of microchannel size and geometry on neurite growth

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has been carried out in polydimethylsiloxane (PDMS) [14] and alginate [15] gels. Furthermore, only a few scaffolds incorporating multiple channels have been reported [16,17].

As the role of a synthetic scaffold is to accelerate nerve repair across the injury site, a number of strategies' have been explored to increase axonal growth within these devices. These include drug delivery [18], addition of cellular components [19,20], electrical stimulation [21,22], and topography [23]. The latter has been primarily studied in the context of flat lithographically patterned substrates [10,23–25] limited to macroscopic rolling for experiments *in vivo* [12]. Consequently, despite the abundance of literature illustrating the growth promoting effects of topographical features [10], nerve guidance channels approved for clinical use remain limited to a simple round geometry [26].

We hypothesize that the current materials processing techniques have been limiting the geometries of neural guidance channels and propose an alternative approach to fabrication of these devices. We employ thermal drawing process (TDP) [27,28] commonly used in fiber photonics and recently applied to neural probe design [29,30], to produce flexible, biocompatible polymer-based neural scaffolds with a variety of geometries and dimensions. The versatility of TDP enabled us to produce, with accuracy, an array of neural scaffolds with cylindrical and rectangular core geometries and dimensions approaching those of single fascicles (inner sizes 50–200 μm and lengths up to tens of centimeters). Furthermore, TDP allowed for direct integration of microgrooved topography within the structure of scaffold channels. The palette of fiber-based neural scaffolds allows for the first time, a detailed *in vitro* analysis of channel size, geometry, and surface topography for potentially promoting nerve regeneration.

2. Materials and methods

2.1. Fiber scaffold fabrication

Fiber scaffolds were produced using the thermal drawing process (Fig. 1). Bulk polymer materials were annealed in a vacuum at 105 °C for one month prior to fabrication. Macroscale polyetherimide (PEI, Tg = 216 °C) slabs or cylinders were machined to include the desired channel geometries (round, square, or grooved). If needed, machined PEI slabs were consolidated in a pneumatic heated press at 240 °C to form the final preform (Fig. 1A). Preforms were mounted onto a vertical draw tower, (Fig. 1B) and drawn at 325 °C producing hundreds of meters of fiber. Increasing stress during fiber drawing allowed for tuning of channel dimensions from 300 μm to 40 μm.

2.2. Cross sectional imaging

Fiber scaffolds were sealed in an epoxy matrix and mechanically polished with a semiautomatic polisher/grinder (RotoPol-1, Struers). Cross sections of the fibers were then imaged using an optical microscope (Axioskop, Carl Zeiss Inc.) in transmission mode (Fig. 1).

2.3. Mechanical properties

The axial spring constant for round and rectangular PEI fibers with an elastic modulus of 104.8 MPa and a fiber length of 10 mm (Supplementary Information) was calculated according to Kozai et al. [31].

2.4. Isolation of dorsal root ganglia

All animal procedures were approved by the MIT Committee on Animal Care and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Primary dorsal root ganglia (DRGs) were isolated from Sprague Dawley neonatal rats postnatal day 1 (P1; Charles River) as previously reported [32]. The spinal cord was exposed and isolated using a posterior approach. Individual DRG explants were collected from the lumbar region, trimmed of nerve roots and connective tissue,

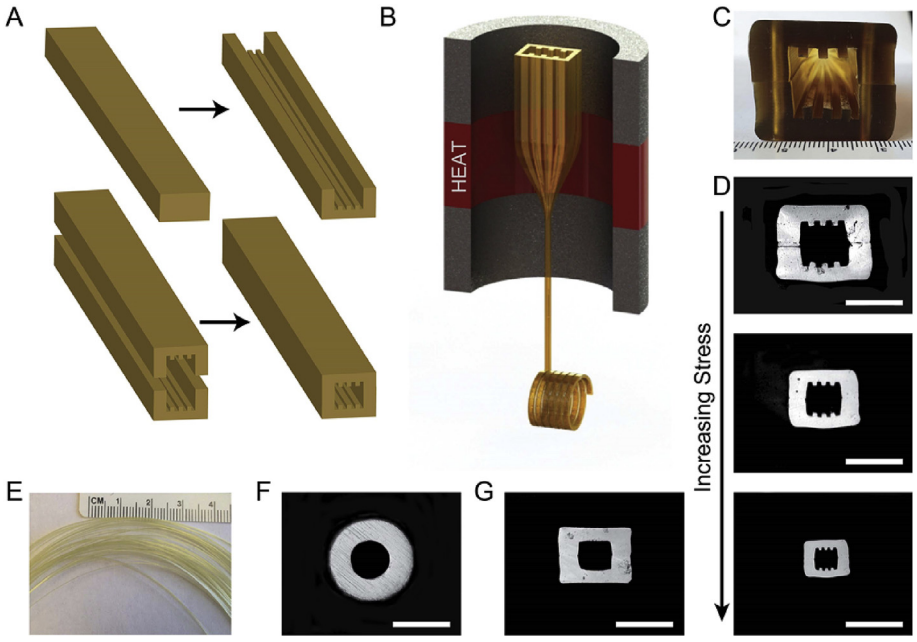

Fig. 1. The thermal drawing process and fiber cross-sections for neural guidance channels. (A) A macro scale preform was fabricated by first machining features into PEI slabs, consolidated, and (B) loaded within the draw tower and heated above the glass transition temperature. (C) Cross-section of machined preform prior to consolidation. (D) The draw down ratio is increased with increasing stress applied via the capstan, yielding a range of fiber dimensions and (E) hundreds of feet of micro scale fiber while maintaining the geometry of the original preform. Scaffolds were also made with (F) round and (G) square geometries. Scale bars = 200 μm.