

A Polymer Foam Conduit Seeded with Schwann Cells Promotes Guided Peripheral Nerve Regeneration

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ABSTRACT

Alternatives to autografts have long been sought for use in bridging neural gaps. Many entubulation materials have been studied, although with generally disappointing results in comparison with autografts. The purpose of this study was to design a more effective neural guidance conduit, to introduce Schwann cells into the conduit, and to determine regenerative capability through it in an *in vivo* model. A novel, fully biodegradable polymer conduit was designed and fabricated for use in peripheral nerve repair, which approximates the macro- and microarchitecture of native peripheral nerves. It comprised a series of longitudinally aligned channels, with diameters ranging from 60 to 550 microns. The luminal surfaces promoted the adherence of Schwann cells, whose presence is known to play a key role in nerve regeneration. This unique channel architecture increased the surface area available for Schwann cell adherence up to five-fold over that available through a simple hollow conduit. The conduit was composed of a high-molecular-weight copolymer of lactic and glycolic acids (PLGA) (MW 130,000) in an 85:15 monomer ratio. A novel foam-processing technique, employing low-pressure injection molding, was used to create highly porous conduits (approximately 90% pore volume) with continuous longitudinal channels. Using this technique, conduits were constructed containing 1, 5, 16, 45, or more longitudinally aligned channels. Prior to cellular seeding of these conduits, the foams were prewet with 50% ethanol, flushed with physiologic saline, and coated with laminin solution (10 $\mu\text{g}/\text{mL}$). A Schwann cell suspension was dynamically introduced into these processed foams at a concentration of 5×10^5 cells/mL, using a simple bioreactor flow loop. *In vivo* regeneration studies were carried out in which cell-laden five-channel polymer conduits (individual channel ID 500 μm , total conduit OD 2.3 mm) were implanted across a 7-mm gap in the rat sciatic nerve ($n = 4$), and midgraft axonal regeneration compared with autografts ($n = 6$). At 6 weeks, axonal regeneration was observed in the midconduit region of all five channels in each experimental an-

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imal. The cross-sectional area comprising axons relative to the open conduit cross sectional area (mean 26.3%, SD 10.1%) compared favorably with autografts (mean 23.8%, SD 3.6%). Our methodology can be used to create polymer foam conduits containing longitudinally aligned channels, to introduce Schwann cells into them, and to implant them into surgically created neural defects. These conduits provide an environment permissive to axonal regeneration. Furthermore, this polymer foam-processing method and unique channeled architecture allows the introduction of neurotrophic factors into the conduit in a controlled fashion. Deposition of different factors into distinct regions within the conduit may be possible to promote more precisely guided neural regeneration.

INTRODUCTION

ENORMOUS EFFORT HAS BEEN DEVOTED to the generation of a synthetic guidance conduit for the repair of peripheral nerve defects.¹⁻⁸ Biosynthetic conduits provide an attractive alternative in bridging extensive nerve defects that are too lengthy to repair with autografting techniques; the current experimental approach employs the use of cadaveric nerve allografts, necessitating undesirable long-term immunosuppressive therapy.⁹ Synthetic conduits are also under investigation for the repair of short peripheral nerve defects, where the goal is to promote superior clinical outcomes to those currently achievable with autografting.

Biological or biocompatible synthetic materials have been employed for decades to bridge neural defects.¹⁰⁻¹⁶ The entubulation model has been extensively examined, where proximal and distal stumps of a severed nerve are inserted into either end of a hollow tube; many of the molecular and cellular events that occur at the regenerating nerve endings have been elucidated in this model.¹³ Investigators hope to exploit the properties of biomaterials to promote these regenerative events more thoroughly.

Biocompatible silicones and other nonbiodegradable inert materials have been successfully utilized to bridge short neural defects. The presence of lumen-occupying substances, particularly extracellular matrix (ECM) components, lengthens the span able to be crossed by regenerating axons.^{3,7} The presence of nerve growth factor (NGF), other neurotrophic agents,¹⁷⁻²³ and Schwann cells²⁴⁻²⁷ has also increased the regenerative response through these conduits. Bridging neural defects with autogenous materials has been modestly successful; skeletal muscle basal lamina (SMBL) grafts have shown promise in bridging longer peripheral nerve defects,²⁸⁻³⁰ possibly because their complex basal lamina architecture promotes robust axonal extension. Most recently, nerve defects have been bridged with biodegradable hollow conduits, following the observation that neural regenerates encased by foreign material over extended periods tend to develop compression syndromes.³¹⁻³³

In this study, a fully biodegradable polymer conduit has been fabricated for bridging peripheral nerve defects. The design simulates both SMBL and autograft architecture, containing a series of longitudinally aligned channels. These channels, with finely controlled diameters and hole positions, provide an increased surface area for Schwann cell migration and adherence, as well as for growth cone elongation at the regenerating axon tips. The polymer material consists of a high-molecular-weight copolymer of lactic and glycolic acids (PLGA) (MW 130,000), which is well tolerated *in vivo*, and whose degradation time can be controlled by altering the ratio of the two monomers. The degradation mechanism is similar to known physiological processes: the polymer is broken down by simple hydrolysis, and the resultant products removed by the Krebs's cycle.³⁴ This polymer has proven effective as a drug delivery vehicle,^{34,35} adding to its potential value as a neural conduit material.

Schwann cells were previously demonstrated to adhere well to PLGA film surfaces.³⁶ Consequently in this study, an increased number of adherent Schwann cells were expected in a multichannel conduit in comparison with a single-lumen conduit, because of the increased luminal surface area. These Schwann cells were introduced into the conduits *in vitro* during the preimplantation dynamic seeding process.

A small series of animals were implanted with Schwann cell-seeded five-channel conduits. The degree of axonal regeneration through them was comparable to that achieved with autografts.

MATERIALS AND METHODS

Polymer foam processing

A 10% solution (wt/wt) of polylactide-co-glycolide (PLGA) in an 85:15 monomer ratio (Birmingham Polymers, Birmingham, AL) was prepared in reagent grade 99.99% glacial acetic acid (Aldrich, St. Louis, MO) [freezing temperature (T_f) = 16.2°C]. Polymer foam conduits were prepared using a novel injection molding technique, to be described elsewhere.³⁷ The polymer solution was injected under low pressure into a stainless steel mold and cooled to a temperature below the PLGA/acetic acid freezing temperature. After freezing, the polymer foam conduit was demolded and freeze dried in a lyophilizer under 30–40 mTorr vacuum. These processed polymer foams were stored in a dry environment until use.

The channels were created in the polymer conduits by stainless steel wires, which were held in place by stainless steel wire mesh inserts at either end of the mold cavity. Wires had diameters of 60–500 microns, depending on the number and diameter of channels desired. They were positioned in different configurations through the wire mesh cloth, creating different luminal compositions (Fig. 1).

Polymer foam characterization

Cross sectional conduit morphology was assessed using a high-resolution scanning electron microscopy (SEM) (Jeol USA Inc., Peabody, MA, model #FTG 6320). The samples were prepared by gold-palladium sputter coating, then imaged between 130× and 370× magnification.

The foams were characterized using gel permeation chromatography, differential scanning calorimetry, and nuclear magnetic resonance spectroscopy; the results are described elsewhere.³⁷

Dynamic seeding of Schwann cells into polymer foam conduits

Schwann cells were isolated from neonatal Fisher rats using previously described methods.^{36,38} The cells were expanded for up to 12 weeks in culture, and then either used or frozen for later use.

Cell seeding was carried out in three stages. First, the conduits were pre-wet in a room temperature bioreactor flow loop by first pulsing a 50% ethanol solution through the polymer conduit and then flushing overnight with physiologic saline; the ethanol solution allowed the hydrophobic polymer pores to be wet by aqueous-based solutions and suspensions. Second, the polymer conduit was laminin-coated in the room temperature bioreactor flow loop by a 10 $\mu\text{g}/\text{mL}$ laminin solution, which circulated for 4 h; the laminin coating promotes the adherence of Schwann cells to the polymer surfaces. Third, a suspension of Schwann cells (5×10^5 cells/mL) was pumped in a closed bioreactor loop (Fig. 2) at 1 mL/min for 4 h through the laminin-coated polymer conduit; this step was conducted in a 5% CO_2 , 37°C incubator environment. Qualitative assessments of Schwann cell adherence were made by a previously described dynamic flowing tetrazoleum conversion assay (MTT).³⁹ After 4 h of exposure to MTT, the polymer conduits were inspected grossly for the presence of a dark purple tetrazoleum product produced by live cells only.

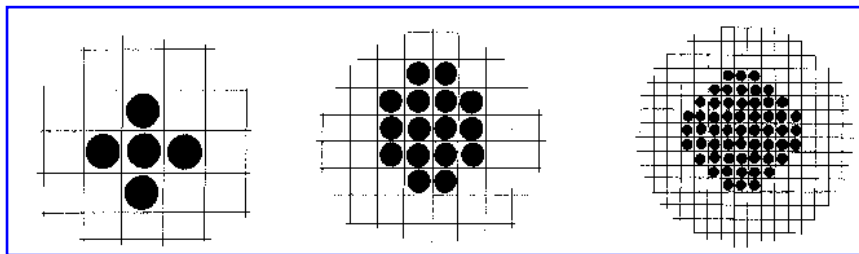


FIG. 1. Schematic demonstrating the appearance of the wires (black circles) threaded through wire cloth in different configurations. Shown are a 5-channel insert, 16-channel insert, and 57-channel insert.

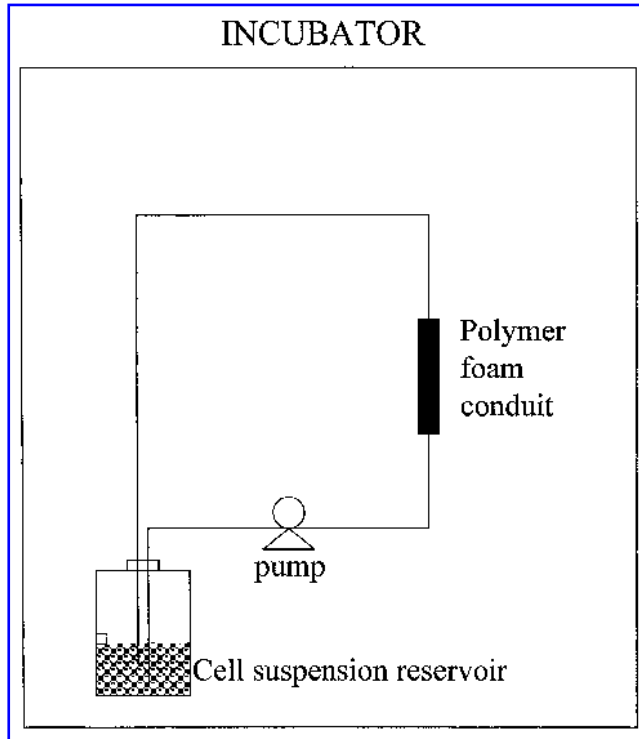


FIG. 2. Bioreactor flow loop.

In vivo regeneration studies

Ten Fisher rats underwent microsurgical removal of a section of the left sciatic nerve, followed by conduit or autograft repair. Briefly, after induction using inhalational methoxyfluorane anesthesia, animals were shaved and sterilely prepped. An incision was made over the left hindlimb, and the gluteal musculature was divided to expose the sciatic nerve. For the conduit repairs, the nerve was sharply transected, allowed to contract, and sharply transected again to leave a 7-mm gap between cut ends. For autografts, a 7-mm section of the nerve was marked, sharply transected at both ends, and rotated so that the distal graft met the proximal stump and the proximal graft met the distal stump. The conduits or the autografts were secured into place using two to three 10-0 nylon sutures at both ends, and the incision was closed in layers. Animals were allowed to recuperate, and were given food and water *ad libitum*. Animal Care and Use Committee guidelines were followed. Autotomy was prevented or minimized by weekly treatment with Bitter Apple taste deterrent (Grannick's, Greenwich, CT) to the left foot.

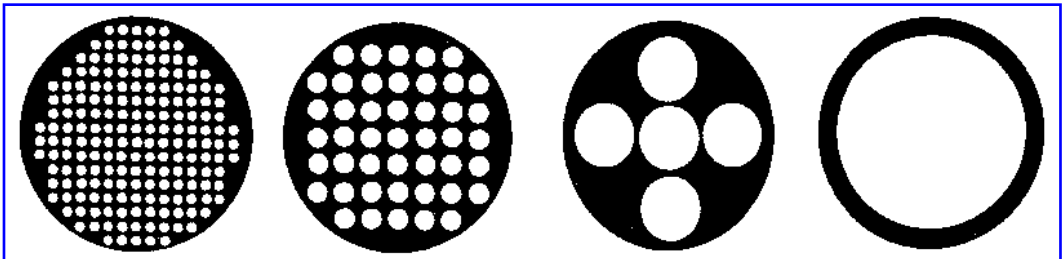


FIG. 3. Schematic illustrating the cross-sectional appearance of polymer conduits after acetic acid removal. Outer diameter was 2.3 mm in all cases. Shown are single channel conduit, 5-channel conduit, 45-channel conduit, and 183-channel conduit.

After 6 weeks, animals were sacrificed via methoxyfluorane overdose, and their sciatic nerves were harvested. The nerves were fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide, and epoxy embedded for histologic analysis. Sections, 1 μm thick, were toluidine blue stained; histomorphometric analysis was performed on the neural regenerate present in the midconduit region.

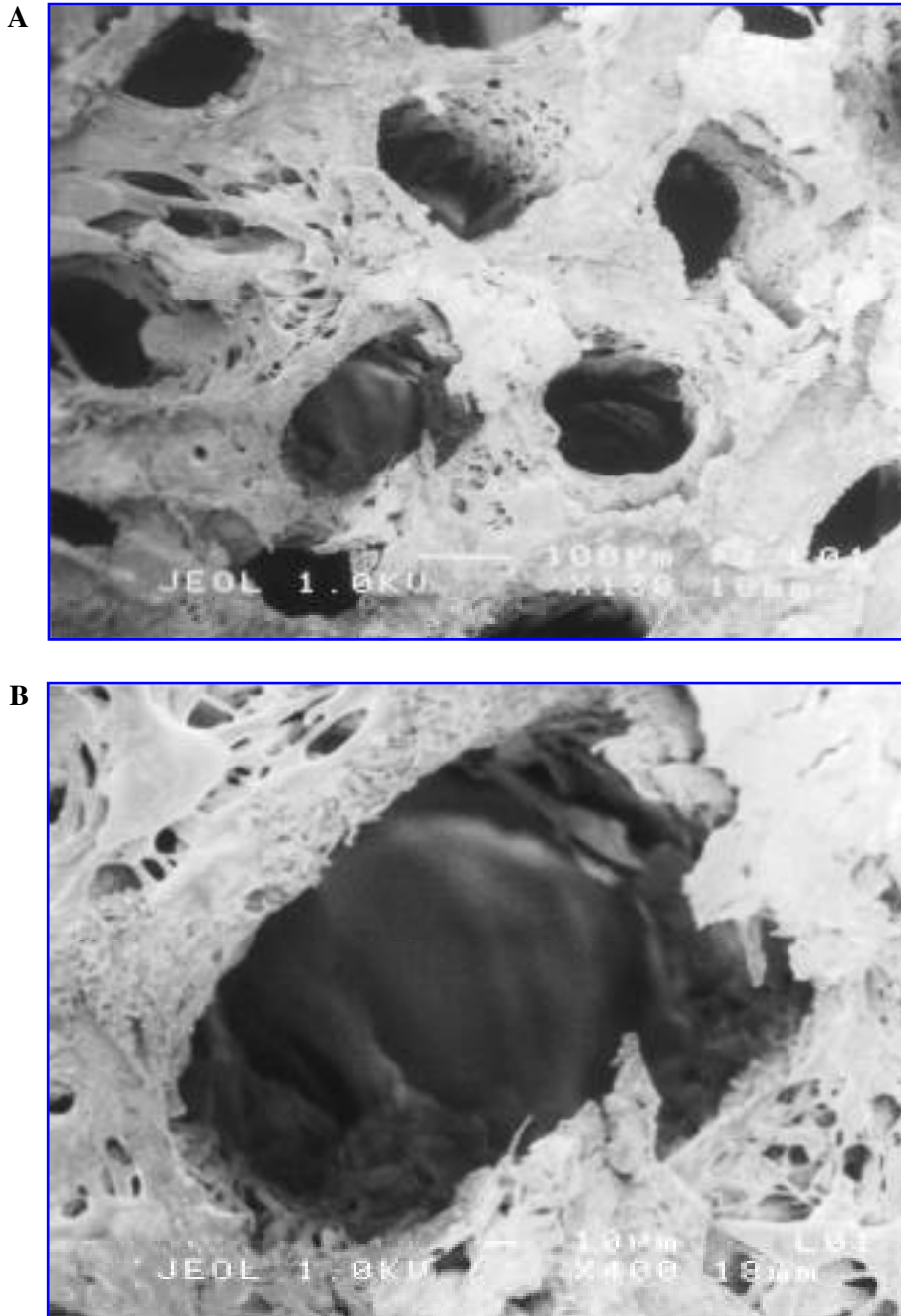


FIG. 4. Electron micrograph of cross section of a 45-channel conduit. **(A)** Magnification: 130 \times . **(B)** Magnification: 400 \times .

RESULTS*Polymer conduit architecture*

Conduits containing five to over one hundred lumens were produced (Fig. 3). The internal diameters of these channels ranged from 60 to 500 microns and corresponded to the diameter of the stainless steel wires used in the molding process. Grossly, the conduits were semirigid, but pliable and able to be manipulated into gentle curves to bridge nerve gaps.

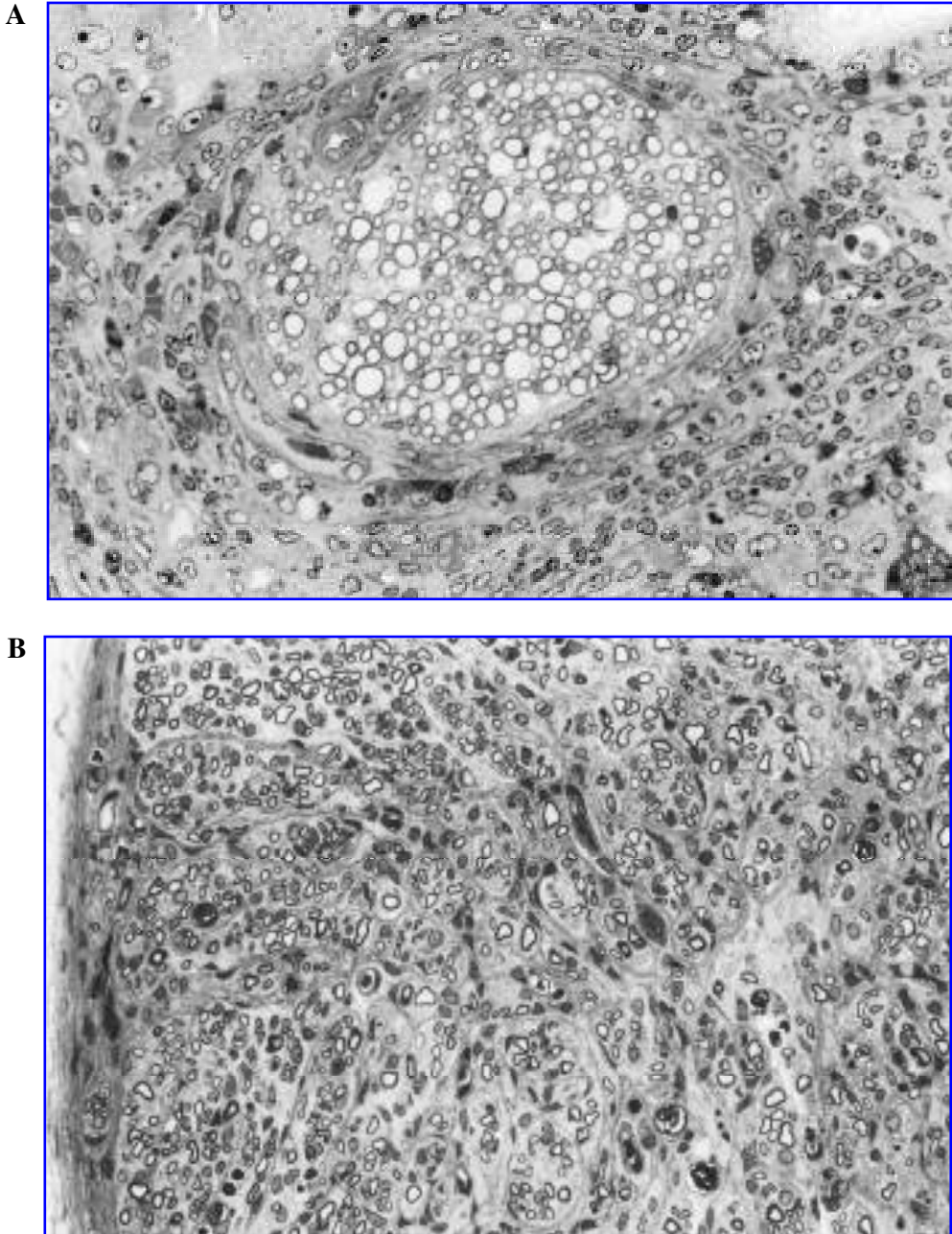


FIG. 5. Histologic section of toluidine blue-stained neural regenerate. (Magnification: 200 \times). (A) One lumen of the polymer conduit. (B) Autograft.

TABLE 1. HISTOMORPHOMETRIC ANALYSIS OF FIVE-CHANNEL CONDUITS AND AUTOGRAFTS

| <i>Type of repair</i> | <i>Fiber width (mm)</i> | <i>Percent neural tissue</i> |
|-----------------------|-------------------------|------------------------------|
| PLGA conduit | 3.73 ± 0.51 | 26.3 ± 10.1 |
| Autograft | 2.54 ± 0.24 | 23.8 ± 3.6 |

Electron microscopy revealed the expected network of pores (Fig. 4). In the absence of significant polymer shrinkage, the pore volume was estimated to be 90% based on a comparison between starting and sublimated weights.

Following the dynamic seeding of Schwann cells, the presence of Schwann cells aligning the channels was verified histologically, using toluidine blue staining of longitudinal sections of the conduits.

In vivo regeneration

Upon harvest, all conduits were intact, with no anastomotic disruptions or fractures along the length of the conduit. Histologic examination of the toluidine blue-stained midconduit sections revealed the presence of a neural regenerate in each of the longitudinally-aligned channels in all experimental animals ($n = 4$) (Fig. 5). As indicated by the histomorphometric analysis results in Table 1, all autografted animals demonstrated regeneration consistent with literature levels for similar gap lengths and survival times. At 6 weeks, the percentage of neural tissue per cross sectional open area through the polymer conduits (mean 26.3%, SD 10.1%) was statistically similar to those through autografts (mean 23.8%, SD 3.6%). However, the mean axon diameter was 3.7 μm , significantly greater than the 2.3- μm mean diameter of control autografts ($p < 0.05$).

DISCUSSION

Based on reported literature studies, conduits with longitudinally aligned channels have demonstrated better regeneration across long peripheral nerve gaps than hollow conduits.^{9,28-30} Although the autografting results have not been functionally or histologically surpassed, skeletal muscle basal lamina grafts and cadaveric nerve allografts have provided encouraging regenerative results. Axonal growth through SMBL grafts improved when the graft is pretreated with Schwann cells in culture before implantation.⁴⁰

In this study, these promising autogenic multichanneled conduit architectures were mimicked in a synthetic polymer configuration. A synthetic system allows the luminal architecture to be application-tailored, providing greater control over the regeneration direction than is possible with autogeneous materials. In addition, *in vitro* luminal introduction of Schwann cells is enhanced in a synthetic conduit in comparison with an autogenous conduit.

The use of PLGA has several advantages. First, the regenerated nerve will not be encased in a foreign material because PLGA is 100% diodegradable; entubulation with nonbiodegradable materials has been shown to lead to compression syndromes over time.³¹⁻³³ Second, PLGA can be tailored to slowly release substances over a protracted period, as demonstrated by its use as a drug delivery vehicle.^{1,34,35} Ultimately this property may be exploited for differential regeneration of distinct neural fiber types.

In a pilot group of animals, regeneration through the polymer conduit has been demonstrated that equals and may surpass regeneration through autografts. The fact that the average fiber diameter in the polymer conduits was significantly greater than that in the autografts suggests that the regenerative environment in the tissue engineered prosthesis may favor regeneration of myelinated ("fast") motor fibers. This finding has broad implications in peripheral nerve surgical repair, because optimal motor function is the ultimate clinical goal.

The total fiber number through the synthetic conduits was low in comparison with autografts because the

five-channeled prostheses had a low open cross-sectional conduit area (20%) in comparison with autografts (50%). Given the quality of the neural regenerate in each channel, however, additional channels within the conduits should lead to a higher overall fiber number.

Ongoing studies in this laboratory continue to address the optimization of cellular seeding into the conduit, and the assessment of functional recovery following autograft or conduit neural repair.

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