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# Axial Microchannel-based Cellularized Nerve Guidance Conduits for Directed Axonal Regeneration

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**Keywords:** Electrospinning; directional channels; Biomaterials; Cellular grafts, rat kinematics.

## 1. Introduction

Peripheral nerve injury (P.N.I.) can be chronic trauma usually resulting from accidents, war injuries and other pathologies, characterized by permanent motor and sensory impairment leading to difficulties in daily life. In clinics across Europe and the U.S. more than 500,000 cases are reported each year, making this one of the major chronic traumas. Treatments for PNI involve autograft, cadaveric allografts and simple hollow conduit tubes, which are the current modalities, rarely restore full pre-injury function, particularly for significant nerve gaps (> 5.0 cm). The performance of patient-derived autografts is superior but is not always feasible. Thus, it is important to explore the synthetic grafts with functional biomaterials, drugs, and material design as cues to enhance axonal regeneration (Perrelle et al. 2022). In this work, we designed cellularized bilayer conduits consisting of directional microchannels. The in-vitro and in-vivo studies showed good biocompatibility and promising regeneration capabilities. Further, the functional recovery in rodent models was studied by leveraging deep learning technique-based video analysis (Sturman et al. 2020). This should help to understand

the regeneration of the axons based on analyzing the behavioral patterns and motor functions in the rodent models.

## 2. Methods

### 2.1 Electrospinning

For electrospinning, 25% (w/v) Poly-4-hydroxyphenethyl 2-(4-hydroxyphenyl)acetate glutarate in Hexafluoro-isopropanol (HFIP) solution was pumped at a controlled rate using the syringe connected with a 23 G needle. An electrical potential was created between the needle and the collector by applying a constant voltage of 20kV to fabricate the fibers. The potential at the collector was altered to obtain the multi-layered tubes with random (rotation speed of 250 rpm) and aligned orientations (rotation speed of 500 rpm) (**Fig 1A**).

### 2.2 Axial microchannels by freeze templating

Gelatin, Chitosan and Collagen (type I) were mixed with EDC-NHS cross-linking agent and frozen unidirectionally in an insulated Styrofoam at  $-135^{\circ}\text{C}$  (Liq. N<sub>2</sub>). After freezing, samples were kept in a  $-20^{\circ}\text{C}$  freezer for 24 hours for cross-linking, thawed (room temperature) and washed with a copious amount of water.

### 2.3 Cell studies

Human induced pluripotent stem cells (hiPSCs) were cultured in with various growth factors to promote a Schwann cell progenitor phenotype (human induced Schwann cell progenitors; hiSCP's)) for biocompatibility studies. For the *in-vivo* studies, hiSCP cells were seeded 10 days before the rodent surgeries. All surgical experiments were performed under an approved protocol of the Rutgers Animal Care and Facilities Committee and the Institutional Animal Care and Use Committee (IACUC) (**Fig 1B**).

### 2.4 Kinematic studies for functional assessment

Animals were subjected to perform gait walks, ladder walks, and open field walks to assess the rat kinematics in pre- and post-rodent surgeries. The results were studied using the deep learning-based software DeepLabcut(Nath et al. 2019).

## 3. Results and discussion

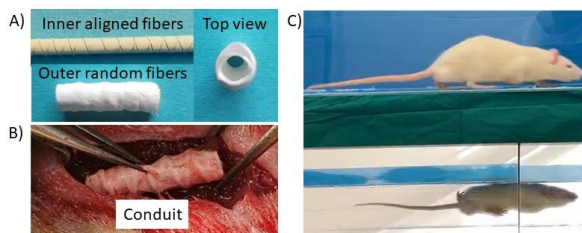
To mimic patient-derived autografts, we fabricated a nerve guidance conduit (NGC) with four unique components: (1) semipermeable walls that permit the transfer of nutrients and waste products, limit cell infiltration, and resorb steadily into the body while being replaced by endogenous tissue; (2) bi-layered walls with unoriented texture on the outside to promote and localize fibroblast and other inflammatory cell deposition to the graft exterior and axially aligned fibers on the inside for axonal growth and alignment; (3) a hydrogel interior patterned with microchannels

and supplemented with growth factors to support ingrowth of axons; and 4) human Schwann cells which reside within the lumen and support axon ingrowth via myelination, guidance cues, and growth factor secretion. The properties of the nerve guidance conduits shown in **Table 1**. The porosity of the electrospun fibers and the hydrogels with unidirectional channels was found to be ideal for facilitating the movement of the nutrients and efflux of the waste, creating a favourable environment for the cell proliferation and regeneration of the axons.

**Table 1.** Microchannel-based NGCs properties.

Properties	Fibers (Outer Tube)	Hydrogel (Inner Lumen)
Porosity	~ 40 %	~ 89 %
Diameter	~0.6 $\mu\text{m}$ (Fiber)	~ 80 $\mu\text{m}$ (Pore)
Modulus	-	8.09 kPa
Water absorption capacity	-	20 times

The cell studies revealed good cell viability and the alignment of the cells in the channel direction, which may play crucial role in providing directionality to the regenerating axons. After the implantation of the cellularized grafts, the functional improvement was studied by performing histology and the rat kinematic video analysis (**Fig 1C**). The kinematic studies revealed the changes in the motion and behaviour patterns, indicating post-surgery improvements.



**Figure 1.** A) Photographic image showing the multi-layered nerve guidance conduit tubes with inner aligned and outer random fibers. B) Photograph showing the nerve guidance conduit transplantation. C) Representative image showing the gait walk performed for the rat kinematic studies.

#### 4. Conclusions

Multi-layered nerve guidance conduits can be fabricated by electrospinning from a hydrolytically degradable polymer. Directional freezing can be used to obtain microchannels in the hydrogel in the lumen. Human Schwann cell progenitors generated from induced pluripotent stem cells (hiPSCs) cultured on the NGCs porous hydrogel showed non-significant immunogenicity. Also, the cell orientation during *in*

*vitro* analysis was observed in the direction of the channels, making them suitable grafts for the axonal regeneration based on the physical cues. The *in-vitro* and *in-vivo* studies on the rodent models incorporating these channelized and cellularized NGCs show the potential of promoting PNI repair. Furthermore, deep learning-based video analysis used for the understanding the functional recovery in the rodent model provide the insights into the recovery process involved based on the behavior pattern and motor functions.

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#### Conflict of Interest

The authors declare no conflict of interest.

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