# Aligned Biodegradable Polymer Nano/Micro Filaments for Guided Neurite Extension

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**Introduction:** Filamentous structures that mimic natural extracellular matrix (ECM) morphology may play an important role in promoting nerve regeneration and repair [1-3]. The major structural component of natural ECM is collagen, which is made of a 3D nano-fibril (50-500 nm in diameter) network [8]. Therefore, biocompatible and biodegradable polymer filaments that mimic the natural ECM structure could serve as a promising scaffolds for guided tissue restoration. *In vitro* studies have shown that the neurite outgrowth of nerve stem cells is guided along the direction of polymer fiber orientation [4]. This contact guidance effect is particularly important in nerve regeneration, which requires directional growth. The objective of this study was to electrospin [5-7] nano/micro meter scale aligned biodegradable filaments to fabricate scaffolds that mimic the structural features of natural ECM, and enhance peripheral nerve regeneration.

**Methods:** The PLGA (85:15) (Mw 20,000) solution (15~55% (w/v)) was prepared by dissolving the polymer into a mixture of THF and DMF (1:1) solvent. To generate aligned PLGA filaments, the solution was electrospun onto a high speed rotating metal drum, wrapped with an aluminum foil. A high voltage of 22kV was applied for the electrospinning process. SEM was used to visualize the filaments and quantitative fiber dimensions were obtained from image analysis software ImagePro. The collected nano/micro filaments samples were kept in a desiccator and was sterilized by UV lamp for *in vitro* experiments. Dorsal root ganglion (DRG) neurons was dissected from P4 rat pups, seeded on the filaments and cultured *in vitro* for 1 week. The orientation of neurite outgrowth was visualized by using fluorescence microscope after immunostaining for neurofilament 160. The neurite outgrowth length was quantified by image analysis software ImagePro.

**Results / Discussion:** The concentration of polymer solution and the concentration of additional $ZnCl_2$ significantly affected the morphology of PLGA (85:15) scaffold obtained from electrospinning process.

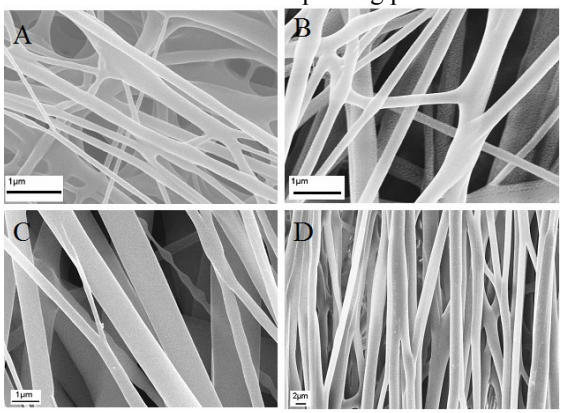


Figure 1. SEM micrographs of electrospun PLGA(85:15) A. 15% PLGA, 4% $ZnCl_2$, B. 25% PLGA, 2% $ZnCl_2$, C. 35% PLGA, D. 55% PLGA.

Aligned PLGA (85:15) filaments with different diameter range were obtained as shown in Fig. 1 (please note the differences in scale bars). The filament diameters are listed in the Table 1. It is obvious that with increasing concentration of the polymer solution, the filament diameter increases. We expect that the neurite extension would be different on these scaffolds with different structural features and these studies are underway now.

Table 1. Electrospun PLGA(85:15) filament diameters

| | 15% PLGA 4% $ZnCl_2$ | 25% PLGA 2% $ZnCl_2$ | 35% PLGA | 55% PLGA |
|---|---|---|---|---|
| Filament diameter | 100-500 nm | 200-800 nm | 300 nm – 1.5μm | 2-3 μm |

As shown in Fig. 2., after 1 week of culturing, the electrospun PLGA (85:15) filamentous scaffold supported neurite extension *in vitro*. And the neuronal processes from the P4 DRGs grow along the PLGA (85:15) filament alignment direction in both cases. It is expected that the neuronal processes would be different on the scaffolds with different structural features. We want to investigate these differences so that to optimize the structural aspect of the scaffold for nerve regeneration. From Fig. 2, the longest neurite outgrowth length on the PLGA (55%) scaffold is 2.9 mm, which is longer than that on the PLGA (35%) scaffold (2.5 mm).

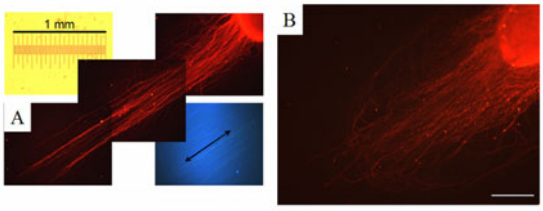


Figure 2. Fluorescence micrographs of cultured P4 rat DRGs. A. on electrspun PLGA (55%) filament (filament pic. on the right bottom corner), B. on electrospun PLGA (35%) filament (scale bar: 0.5 mm).

**Conclusions:** PLGA (85:15) was electrospun into aligned nano/micro fibrous scaffolds to mimic the natural ECM structure and to incorporate the contact guidance concept. The filament diameter and alignment can be tuned by manipulating the processing parameters. The PLGA scaffold supported and guided the neurite extension from P4 rat DRGs *in vitro*. Systematic studies are undergoing to investigate the dependence of neurite outgrowth on the scaffold filament dimensions.

**References:**

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