Effect of Ultrasound on Peripheral Nerve Regeneration Using Asymmetrically Porous PLGA/Pluronic F127 Nerve Guide Conduit

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Statement of Purpose: Restoration with sufficient functional recovery after peripheral nerve injury continues to be a clinical challenge [1]. Transplantation of autologous nerve graft having lesser functional importance has been used for injured peripheral nerve repair as a first line therapy. However, their need of the second surgical step for the extraction of donor nerve, permanent loss of the donor nerve function, limited supply of available grafts, and mismatch between defect nerve and graft nerve dimension are still remained as limitations [2]. Artificial nerve guide conduit (NGC) to bridge the gap between severed peripheral nerve stumps has been widely accepted as an useful alternative that creates a favorable micro-environment for nerve regeneration [3]. Although the NGCs from biodegradable synthetic polymers have been fabricated by many methods, inefficient nutrient permeation into the NGCs caused by their hydrophobic character and residual organic solvents are still remained as limitations for nerve regeneration. Recently, we developed a novel method to fabricate a NGC with the asymmetrical pore structure and hydrophilicity using poly(lactic-co-glycolic acid) (PLGA) and Pluronic F127 by a modified immersion precipitation method [4]. We recognized that the PLGA/F127 (3 wt%) tube can be a good candidate as a NGC from the analyses of its morphology, hydrophilicity, nutrient permeability and model animal study (sciatic nerve defect of rat). In this study, we applied ultrasound at the PLGA/F127 NGC-implanted site transcutaneously in rats to evaluate that the ultrasound can stimulate Schwann cells and thus improve nerve regeneration rate.

Methods: To prepare asymmetrically porous hydrophilized NGCs, PLGA was dissolved in tetraglycol at 60 ^{o}C (10 wt%) and then Pluronic F127 was added in the PLGA solution (5 wt%, PLGA base). Rod-shape alginate hydrogels were fabricated by the injection of 4 wt% alginate solution into 2 wt% $CaCl_2$ solution through a syringe. The prepared alginate gel rod was immersed into the PLGA/F127 mixture solution. In this step, the PLGA/F127 mixture was precipitated on the alginate gel rod. The PLGA/F127 tube was produced after washing the PLGA/F127-coated alginate gel rod in water to remove tetraglycol for 6 hrs and drying in a vacuum oven overnight. Surface and cross-section morphologies of the porous PLGA/F127 tubes were observed by a SEM. The prepared porous PLGA/F127 tubes had an inner diameter of ~ 1.5 mm and wall thickness of ~ 0.4 mm. Sprague-Dawley rats (weight, ~250 g) were used to investigate ultrasound effect on nerve regeneration through the PLGA/F127 tube as a NGC. The PLGA/F127 NGC-implanted animals were received ultrasound at a frequency of 1 MHz and intensity 0.4 W/cm^2 for 2 min with different stimulation patterns. Their nerve regeneration behaviors were compared with the control (PLGA/F127 NGC without ultrasound) and normal nerve by histological observations (Toluidine blue staining and TEM).

Results: It was observed that the PLGA/F127 tube wall has asymmetric column shape by the phase separation between the polymer solution and nonsolvent. The outer surface of the tube has micron pore sizes (~ 50 μm) which can allow vascular ingrowth into the tube wall, and the inner surface has submicron ones (~ 50 nm) which can effectively prevent from fibrous tissue infiltration but allow oxygen and nutrients permeation. At the pre-determined period after surgery in rats, the NGCs were harvested, and the specimens were made from the mid-tubes for morphology analysis. It was observed that the ultrasound has a positive effect on the peripheral nerve regeneration through the PLGA/F127 NGC. Particularly, group 4 (every week ultrasound stimulation) showed better nerve regeneration behavior than other groups (Fig. 1). From this study, we could conclude that the ultrasound stimulation may be an efficient tool to stimulate peripheral nerve regeneration using asymmetrically porous hydrophilized NGC.

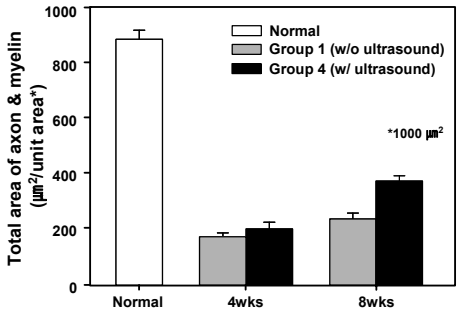

Figure 1. Comparison of total area of axon and myelin sheath in the NGCs at the middle section after 4 and 8 weeks implantation (n = 3).

References:

1. Schlosshauer B, et al. Brain Res. 2003;963:321-326.
2. Wang S, et al. J Biomed Mater Res. 2003;66:522-531.
3. Glück T. Arch Klin Chir. 1880;25:606-616.
4. Oh SH, et al. Biomaterials. 2008;29:1601-1609.

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