Peripheral Nerve Regeneration by Asymmetrically Porous PLGA/Pluronic F127 Nerve Guide Conduit

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Introduction

The causes of nerve injury have been referred to traffic accidents, tumor damages, viral infections and the side effects of neurosurgery [1]. Direct reconnection of proximal and distal nerve stumps, and transplantation of autologous nerve graft have been used for injured nerve repair. However, the former can be applied only slight or small defect and the latter also has some problems, such as the need of the second surgical step for 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 [2]. Artificial nerve guide conduit (NGC) to bridge the gap between severed nerve stumps is widely accepted as a useful alternative that creates a favorable micro-environment for axonal regeneration, is free of the limitations of natural grafts, and requires no immunosuppressants [3]. Although the NGCs have been fabricated by many methods such as immersion precipitation, casting and extrusion, their permeability (which should prevent fibrous scar tissue invasion but allow nutrient supply) and residual organic solvent are still remained as limitations. In this study, we developed a novel method to fabricate a NGC with the porosity of submicron pore sizes (to prevent fibrous tissue infiltration) and hydrophilicity (for effective oxygen and nutrient permeation) using poly(lactic-co-glycolic acid) (PLGA) and Pluronic F127 by a modified immersion precipitation method designed by our laboratory [4]. The morphology, mechanical strength, hydrophilicity and model nutrient permeability of the fabricated NGCs were investigated. The *in vivo* animal study to investigate nerve regeneration behavior using a rat model was also examined.

Experimental

To prepare hydrophilized microporous NGCs, PLGA/Pluronic F127 (0 ~ 5 wt%, PLGA base) was dissolved in tetraglycol. The alginate hydrogel rod crosslinked by 2 wt% $CaCl_2$ solution was immersed into the PLGA/F127 mixture solution. The PLGA/F127 mixture was precipitated outside the alginate gel rod by the diffusion of water from the alginate gel rod into PLGA/F127 mixture in tetraglycol. The PLGA/F127 tube was produced after washing the PLGA/F127-coated alginate gel rod in water and drying. The PLGA/F127 tubes were evaluated by the characterization of their surface and cross-sectional morphologies, mechanical strengths, nutrient permeability, etc. To estimate nerve regeneration through the PLGA/F127 tube as a NGC (length, 12 mm; inner diameter, 1.5 mm), *in vivo* animal study was also conducted using a rat model (sciatic nerve defect). The nerve regeneration was evaluated by histological and immunohistochemical observations (H&E, Toluidine blue, anti-neurofilament staining and TEM), and electro-physiological evaluation (compound muscle action potential (CMAP)).

Results and discussion

It was observed that the outer surface of the tube prepared by a modified immersion precipitation method has micron pore sizes (~ 50 μm) which can allow vascular ingrowth into the tube wall, however, the inner surface has submicron ones (~ 50 nm) which can effectively prevent from fibrous tissue infiltration but allow oxygen and nutrients permeation (Fig. 1). The inner diameter and wall thickness of tubes could be easily controlled by adjusting the diameter of alginate hydrogel rod and immersion time, respectively. From the animal study, it was observed that the axons were continuously and straightly grown through the NGCs without the collapse of the tubes with time. The axons were reached into the distal stump at 4 wks (Fig. 2). The hydrophilized PLGA/F127 (3 wt%) tube has a better nerve regeneration behavior than the hydrophobic PLGA tube (Fig. 3), probably due to the effective permeation of nutrients and oxygen. It was recognized that the hydrophilized PLGA/F127 (3 wt%) tube can be a good candidate as a NGC from the analyses of its morphology, mechanical strength, hydrophilicity, model nutrient permeability and nerve regeneration behavior.

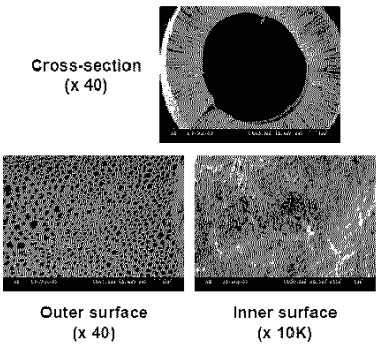

Figure 1. SEM photographs of PLGA/F127 (3 wt%) tube as a hydrophilized NGC.

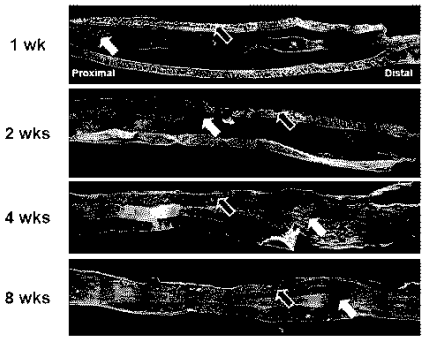

Figure 2. Longitudinal sections of the regenerated nerve through PLGA/F127 (3 wt%) tube (anti-neurofilament staining, x 4; white arrow, regenerated nerve; black arrow, NGC).

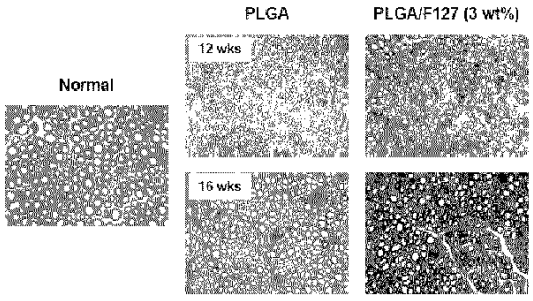

Figure 3. Light micrographs of semi-thin section showing myelinated axons at mid-tube (12 and 16 wks after implantation; Toluidine blue staining; x 1,000).

Acknowledgment

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References

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