Development of Surface Modified Polymeric Conduits for Nerve Regeneration

Metin Uz[1], Anup D. Sharma[1], Donald S. Sakaguchi[1], and Surya K. Mallapragada[1]
[1] Iowa State University

Statement of Purpose: Severe damage to the peripheral nervous system (PNS) may result in malfunction of sensory and/or motor properties, leading to loss of communication pathways between the brain and muscles or organs. Although the PNS does possess the capacity for spontaneous nerve regeneration, it may not be sufficient to bridge large peripheral nerve gaps (>10 mm). At this point, bioengineered artificial nerve conduits have been proposed as a promising strategy for the regeneration of damaged nerves. (Rutkowski, Miller et al. 2004, Oh, Kim et al. 2013, Tang, Zhu et al. 2013). In this study, surface modified porous nerve conduits with longitudinal micro-patterns, based on Poly-L-Lactide (PLLA) was developed to address the problem of bridging large peripheral nerve gaps. The porous structure was created to provide sufficient diffusion of nutrients through the conduit matrix enhancing the nerve growth. The longitudinal micro-patterns and surface modification through gradient nerve growth factor (NGF) loading on the inner surface of conduit lumen was used to stimulate the cellular attachment and guided nerve growth. Besides the gradient NGF loading on the membrane surface, providing initial burst NGF release, the NGF encapsulated in amphiphilic and biodegradable polyanhydride (PAH) microparticles were also distributed in PLLA matrix to manipulate and provide sustained NGF release. Throughout this study, the efficiency of developed conduits in terms of in vitro NGF release profiles and neurite extension and outgrowth against PC12 cells were evaluated.

Methods: NGF encapsulated PAH (50%,1,6-bis-(p-carboxyphenoxy)hexane (CPH), and 50%,1,8-bis-(p-carboxyphenoxy)-3,6-dioxaocatane (CPTEG)) microparticles (NGF/PAH) were formed by spray drying. NGF/PAH distributed PLLA membranes with different pore structures were prepared by dry wet phase inversion method through the casting of polymer solution, containing 10% PLLA and varying amounts of NGF/PAH micrparticles and Pluronic F127 (pore forming agent), on a micro-patterned silicon wafer support. The obtained membranes were coated with nerve growth factor (NGF) through differential adsorption to create NGF gradients on the membrane surface. The conduits were formed by rolling the membranes such that the micro patterned side formed the inner lumen to the conduits. The cumulative NGF release from conduits in PBS buffer (pH 7.4) at 37 °C was monitored with respect to time. To assess NGF bioactivity, the influence of the conduits on PC12 cells in terms of neurite extension and outgrowth were investigated.

Results: The SEM images indicated that different pore structures were obtained by changing the Pluronic F127 concentration in the polymer casting solution (Figure 1.). It was observed that the prepared conduits kept their shape, pore and pattern structure after 10 days of incubation in cell culture medium.

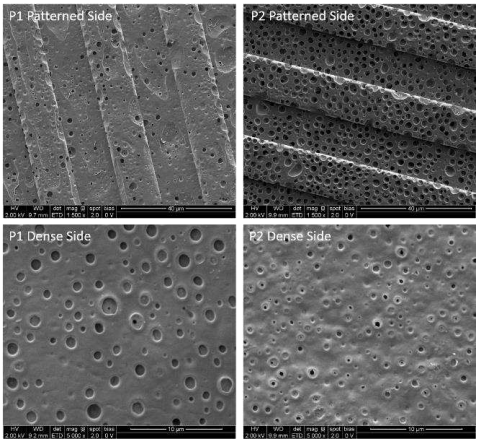

Figure 1. SEM images of prepared membranes with different pore structures. P1: 50% PluronicF127 content. P2: 80% PluronicF127 content.

The NGF loading and release studies demonstrated that different pore structures did not affect the NGF loading significantly whereas it caused slight difference in release rate. Cumulative NGF loading of ~1500 ng/cm^2 was seen from the membranes in 48h of incubation. At the end of 10 days, ~120 and ~80 ng of NGF was released from the membrane surfaces P1 and P2, respectively. The NGF gradient on the surface was assessed by measuring the different released amounts of NGF from different sections of membranes and conduits. The released NGF amount from the proximal section was ~ 32 ng whereas it was ~25 ng/mL for the distal section at the end of 24h. The influence of the developed conduits on neurite formation and outgrowth of PC12 cells was also investigated.

Conclusions: The conduits kept their pattern and pore structure for a long time. High NGF loadings followed by burst gradient NGF release from the surface and sustained release from the NGF/PAH microparticles distributed in the matrix was observed. The membranes provided a proper environment and support for the attachment and directed growth of the cells. These conduits will be further tested in vivo in future work.

References:

Oh, S. H., J. R. Kim, G. B. Kwon, U. Namgung, K. S. Song and J. H. Lee (2013). Tissue Engineering Part C-Methods **19**(3): 233-243.

Rutkowski, G. E., C. A. Miller, S. Jeftinija and S. K. Mallapragada (2004). J Neural Eng **1**(3): 151-157.

Tang, S., J. X. Zhu, Y. B. Xu, A. P. Xiang, M. H. Jiang and D. P. Quan (2013). Biomaterials **34**(29): 7086-7096.