Physical and Chemical Guidance of Axons Using Aligned Conducting Polymer Nanotubes

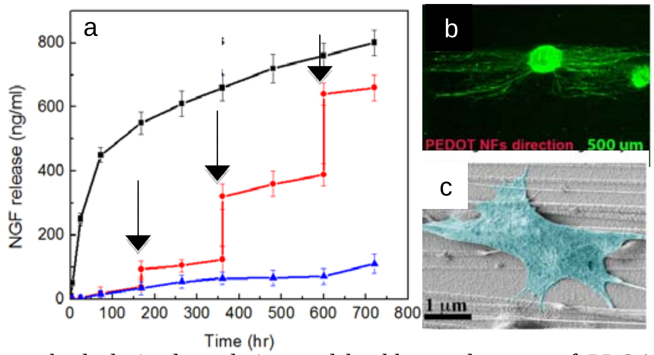
Guang Yang[1],Mohammad Reza Abidian[1,2,3]

[1]Departments of Bioengineering, [2]Chemical Engineering, [3]Material Science and Engineering, Pennsylvania State University,

Statement of Purpose: Nerve injury in both central and peripheral nervous system is a major health problem. Spontaneous axonal regeneration is limited to small lesions within the injured peripheral nervous system and is actively suppressed within the central nervous system [1]. Axons are guided along specific pathways by gradients of attractive and repulsive chemical and physical cues [2]. To understand the effect of guidance cues on axon growth rate, development of in vitro platforms that are capable of producing precisely controlled shape guidance cues is essential. Conducting polymers have been widely used in biomedical applications, in particular, for drug delivery systems and neural interfaces. Conducting polymers have the ability to response to electrochemical oxidation/reduction by changing their color, conductivity, wettability, and volume [3]. Previously we developed a novel method for fabrication of randomly oriented conducting polymer nanotubes for controlled release of an anti-inflammatory drug [4]. We hypothesize that the aligned conducting polymer nanotubes will provide both physical and chemical guidance for axonal regeneration.

Methods: We have developed a novel method for fabrication of multifunctional aligned conducting polymer nanotubes for axonal regeneration. The fabrication process involves (1) electrospinning of nerve growth factor (NGF) loaded poly(lactic-co-glycolic acid)(PLGA) nanofibers (PLGA NFs) on a rotating gold substrate (angular velocity ~1200 RPM) to create aligned PLGA-NGF nanofibers; (2) Electrochemical polymerization of conducting polymer polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene) (PEDOT) on the gold substrate and around the aligned PLGA-NGF NFs to create NGF-loaded aligned PPy and PEDOT nanotubes (PPy NTs and PEDOT NTs). Electrochemical deposition of PEDOT was carried out using Autolab PGSTAT302N by an applied current density of 0.5 mA/cm2. Surface morphology and electrical properties of the biodegradable NFs and conducting polymer NTs were characterized using scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS), respectively. NGF release experiment was carried out by applying 1 V for 5 times. To evaluate the biocompatibility of aligned PEDOT NFs and determine the role of physical and chemical guidance cues, primary dorsal root ganglion (DRG) explants and PC12 cells from rats were cultured on the substrates.

Results: SEM images revealed that the diameter of aligned core PLGA nanofibers and thickness of aligned PEDOT nanotubes were 100 ± 23 nm and 30 ± 5 nm, respectively. EIS data demonstrated that impedance of gold substrate decreased about 2 orders of magnitude after deposition of PEDOT nanotubes. As illustrated in Fig. 1a, we found a release burst of 50% after 90 hr for PLGA NFs (black squares), which might be explained by fast



hydrolytic degradation and backbone cleavage of PLGA NFs[35]. Addition of the PEDOT coating around the NGF-loaded PLGA NFs dramatically slowed down the release rate (blue triangles). Under this condition, less than 10% of the NGF was released after 720 hr. In other words, in the absence of any actuation, more than 90% of NGF remained trapped inside the PEDOT NTs. In order to release the entrapped NGF in a controlled fashion, we actuated PEDOT NTs by applying a bias voltage of 1 V for 5 times at three specific time points of 170, 360, and 600 hr. Fig. 1a illustrates the effect of this actuation on the release profile of NGF (vertical jumps in this data correspond to the applied electrical stimulation, red circles). PEDOT NFs supported neurite outgrowth from the ganglia in the direction of the PEDOT NFs (Fig. 1b). DRGs culture on substrates with aligned PPy and PEDOT NTs revealed that neurites appeared longer on aligned PEDOT NTs compared to aligned PPy NTs (1250±40 mm for aligned PEDOT NFs and 1110±200 mm for aligned PPy NFs). PC12 cell cultures on aligned PEDOT NTs demonstrated that these NTs provided physical guidance for cell adhesion and proliferation. PC12 cells elongated and axons extended in the direction of NTs (Fig. 1c).

Conclusions: We successfully developed a novel method for fabrication of NFG-loaded aligned conducting polymer nanotubes. Experimental results demonstrated precisely release of NGF due to the electrical actuation of NTs. *In vitro* cell culture experiments showed that conducting polymer NTS were able to provide physical and biochemical guidance for axonal regeneration. In Future, we will design a multifunctional nerve conduit using aligned PEDOT-NGF NTs and we will examine the rate of axonal regeneration in 15 mm nerve gap in rats.

References:

1. P. J. Horner, F. H. Gage, *Nature* 2000, 407, 963.
2. B. J. Dickson, *Science* 2002, 298, 1959.
3. N. K. Guimard, N. Gomez, C. E. Schmidt, *Prog. Polym. Sci.* 2007, 32, 876.
4. M. R. Abidian, D. H. Kim, D. C. Martin, *Adv. Mater.* 2006, 18, 405.