Chitosan-polycaprolactone based miscible polyblend nanofibrous conduit for peripheral nerve regeneration

Narayan Bhattarai, Ashleigh Cooper, Dennis Edmondson, Steve Florczyk, Matthew Leung and Miqin Zhang
Department of Materials Science & Engineering, University of Washington, Seattle, Washington 98195, USA

Statement of Purpose: Severe nerve damage in the peripheral nervous system requires graft insertion to induce nerve regeneration at the trauma site. As nerve autografts offer erratic remediation performance and secondary complications, synthetic conduit alternatives have been actively pursued (Johnson, EO. Injury. 2005; 36 Suppl 4: S24-9). Several Artificial conduits have been made from natural and synthetic bio-polymers to exploit each material's biological and mechanical properties (Leach, JB. Encyclopedia of Biomedical Engineering. 2006; 6:1-10.). Few natural and synthetic polymers have been recently fabricated by electrospinning into nanofibrous conduits for nerve repair (Chew, SY. Adv Funct Mater. 2007;17:1288.1296.). While the advances in artificial nerve regeneration are encouraging, none of developed nerve conduits have matched the performance currently achieved by autografts. The limitations rest on the unsatisfactory mechanical or biological properties of artificial nerve conduits which often manifest in structural collapse, material swelling, early resorption, and release of cytotoxic degradation products (Doolabh, VB. Rev Neurosci. 1996; 7: p. 47-84).

In our research, we develop and evaluate a more effective biohybrid neural guidance conduit made of electrospun natural-synthetic polymeric nanofiber comprised of miscible blended chitosan and poly(ε-caprolactone) (PCL). The design combines the technological advances in biocompatible polymers and nanotechnology to produce nanofibrous matrices with significantly improved mechanical and biological properties. Although, chitosan is a well known biodegradable, nonantigenic, and biocompatible natural polymer widely used widely in biotechnology and medicine, it is mechanically weak, and alone it is unable to retain its structural integrity in aqueous environments. The complementary polymer, PCL, is commonly found in tissue engineering applications due to its structural and mechanical stability (Hollister, SJ. Nat Mater. 2005;4: p. 518-524). PCL has limited cell affinity. The well-blended chitosan-PCL nanofibrous matrix that integrates the favorable biological properties of chitosan and mechanical properties of PCL is expected to significantly improve material properties, while providing a stable, nurturing environment for the regeneration of peripheral nerves.

Methods: The optimal blend solution of chitosan-PCL for the electropsinning was found in the mixed solvent of trifluroacetic acid and trifluroethanol. Conduits with thicknesses of 0.2–1.0 mm, inner diameters of 1–5 mm, and lengths of 5–15 cm were fabricated by collecting the electrospun fibers on stainless steel rods with varying thickness and lengths (Bhattarai, N. Adv Mater. 2009; 21: 2792-2797). The structural integrity and, mechanical and biological properties of the nanofibrous conduit were evaluated and compared to control conduits made of synthetic poly (lactide-co-glycolide) (PLGA) and collagen. The cellular compatibility of the nanofibers with neuronal cells for the nerve regeneration was assessed by seeding Schwann and neuron-like PC12 cells. Nerve conduits were also implanted in a critical-size sciatic nerve defect in rats for a month and histological analysis of the explants was carried out.

Results: The nerve conduits made of miscible polyblend nanofibers of chitosan-PCL exhibited excellent mechanical properties and biological activity unmatched by commonly available nerve guide materials (Figure 1). Neuronal cells adhered and proliferated well on the conduit, and demonstrated neurite outgrowth. The in vivo implantation of the nerve conduit also demonstrated that the conduit micro-structures provided the favorable environment to regenerate the nerve fibers.

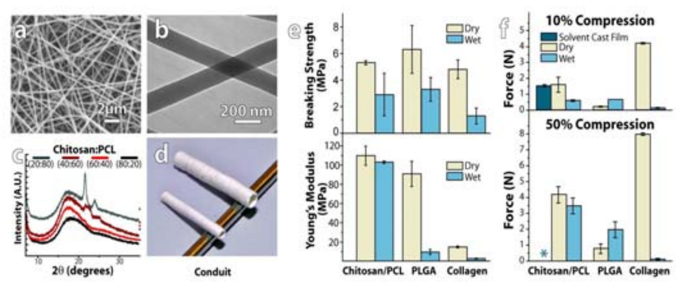

Figure 1. Structural and mechanical properties of nanofirous constructs; (a) SEM image of chitosan-PCL (40/60) nanofibrous matrix. (b) TEM micrograph of nanofibers with varying component mixture ratios; the 40/60 blend showed limited crystallinity indicating increased miscibility. (d) Optical image of fabricated chitosan-PCL conduits with varying internal diameter. Tensile (e) and compressive (f) tests of nanofibrous tubular constructs at both dry and wet states. PLGA and collagen nanofibrous conduits, and solvent casting chitosan-PCL conduit served as controls for comparison study.

Conclusions: A chitosan-PCL miscible blend nanofibrous matrix, which capitalizes on the favorable biological and mechanical properties of chitosan and PCL, respectively, is designed to address the limitations of current nerve guide conduits, while providing a stable, nurturing environment for nerve regeneration applications in very cost effective way.