Control of porosity of braided nerve conduits for peripheral nerve regeneration

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Statement of Purpose: Use of synthetic nerve tubes in peripheral nerve regeneration is currently limited to a critical nerve gap (<4 cm). Exsisting nerve tubes lack the flexibility and desired mechanical behavior required for regeneration across longer nerve gaps. Our previous work suggested that braiding the conduits could impart flexibility and kink resistance which is a unique feature required over longer gaps [1]. Our previous work also showed that conduit porosity can effect nerve regeneration [2]. Braided nerve conduits are mechanically superior but they do pose the problem of scar tissue infiltration due to their macro-pores. In this study, we focused on controlling the porosity of the braided nerve conduits by covering its macro-pores with two different types of outer hydrogel coatings. The conduits were then evaluated in a 1 cm rat sciatic nerve injury model for 6 weeks and 16 weeks to achieve nerve regeneration without any scar tissue infiltration.

Methods: Braided conduits were fabricated by extruding E1001(1K) tyrosine-derived polycarbonate into thin polymer fibers, which were then braided into conduits with 1.5 mm inner diameter. To control the porosity, braided conduits were coated with fibrin glue (FG) or hyaluronic acid (HA). Conduit, coating morphology and in-vitro coating degradation was investigated by scanning electron microscopy (SEM). *In vivo* nerve regeneration was evaluated in the 1 cm rat sciatic nerve model by measuring the compound muscle action potentials (CMAP) at the dorsal and plantar foot muscles. At 6-week and 16-week endpoint, the nerves were harvested, cut into 1µm sections and stained with Toluidine Blue for histomorphometric analysis. Muscles innervated by the sciatic nerve were also harvested and weighed at 16-week endpoint.

Results: E1001(1K) fibers of 70 µm average diameter were braided to fabricate conduits with pore size varying between 15-100 µm. Previously, braided conduits bent at large angles demonstrated flexibility and kink resistance [1]. 6-week in-vitro degradation study of the FG and HA-coated conduits showed that both outer hydrogel coatings were capable of uniformly coating the macropores and were able to maintain their structure till 6-weeks (Fig. 1). Braided E1001(1K) conduits, FG and HA hydrogel coated conduits explants were evaluated for muscle mass recovery and CMAP amplitute to track the reinnervation to distal muscle targets. Histological analysis of the uncoated braided conduits at 6-weeks showed a nerve cable with numerous axon bundles, but with lot of scar tissue infiltration. At 6-week timepoint, HA-coated conduits significantly avoided scar tissue infiltration and lead to an increased density of myelinated axons and a well-defined nerve cable in the conduits (Fig. 2B) compared to the uncoated braided conduits (Fig. 2A). FG-coated conduits lead to extensive fibrous tissue infiltration and minimal regeneration with only a few axons and a fibrous capsule surrounding the conduit (Fig. 2C). Animals treated with HA-coated braided conduits also had an improved electrophysiological recovery compared to the uncoated conduits and the FG-coated conduits. The HA-coated, FG-coated and the uncoated braided conduits will further be evaluated at the 16-week endpoint for histomorphometric analysis and functional recovery.

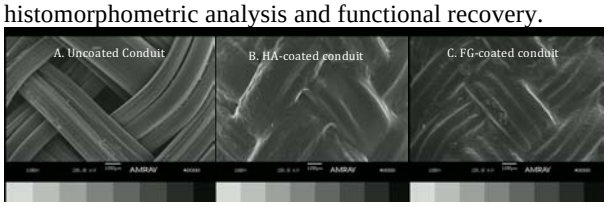

Fig.1 SEM image of a (A) Uncoated braided E1001(1K) conduit (B) HA-coated conduit (C) FG-coated conduit

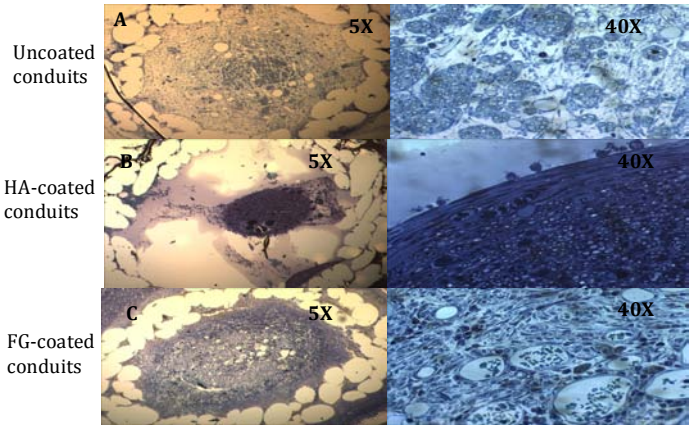

Fig.2. Cross sections of conduits explanted after 6 weeks *in vivo*. (A) The nerve cable in the uncoated braided conduits appeared loosely packed with fibrous tissue infiltration. (B) Nerve cable in the HA-coated braided conduits was densely packed with axons avoiding scar tissue infiltration. (C) FG-coated conduit had extensive scar tissue.

Conclusions: E1001(1K) braided conduits were porous, flexible, and kink resistant. HA-coated braided E1001(1K) conduits showed improved nerve regeneration and were free of infiltrating scar tissue. They performed better compared to the fibrin glue coated conduits. HA-hydrogel coating of the braided conduits appears to be a promising strategy to improve the conduits' *in vivo* performance. 16-week histomorphometric analysis, muscle recovery and electrophysiological evalauations are in process. This result will guide our future studies that aim at the design of synthetic nerve conduits capable of supporting nerve regeneration over gaps larger than 6 cm.

References:

[1] "Braided tyrosine-derived polycarbonate nerve conduits for peripheral nerve regeneration", Basak Clements et al.; TERMIS 2011

[2] "Enhanced femoral nerve regeneration after tubulization with a tyrosine-derived polycarbonate terpolymer; effects of protein adsorption and independence of conduit porosity", Mindy Ezra et al.; Tissue Engg Part A, September 6, 2013 (ahead of print).