Mechanically-tunable Extracellular Matrix Hydrogel Scaffold for Use in a Tissue-Engineered Electronic Nerve Interface (TEENI)

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Statement of Purpose: Just under 2 million people live with limb loss in the United States alone [1] which confines them to using prostheses. Peripheral nerve interfaces (PNIs) are one type of device developed to control robotic prostheses. PNIs still face a number of challenges such as long-term signal fidelity because of foreign body response before their widespread use [2].

Our approach is to develop a Tissue-Engineered Electronic Nerve Interface (TEENI) to allow for native tissue regeneration post-implantation enabling intimate contact between regenerating axons and electrodes which may reduce foreign body response. Microfabricated thin-film polyimide-based "thread" electrodes were developed for this project. To support these threads and facilitate axonal regrowth, a composite hydrogel was developed and optimized, as well as a fabrication technique for forming the complete devices.

Methods: Two hydrogel compositions were fabricated from collagen I (3 mg/mL), laminin (1.5 mg/mL), and methacrylated hyaluronic acid (MAHA), with the MAHA concentration varied to tune the mechanical properties of the hydrogels (3 mg/mL (low) or 6 mg/mL (high)). The hydrogel components were mixed at appropriate concentrations and incubated at 37 °C for 30 minutes followed by 5 minutes photocrosslinking under UV light.

Shear and compressive moduli of hydrogels and native rat sciatic nerves were characterized. Shear storage and loss moduli were determined at an amplitude of 0.5% strain over an angular frequency range of 0.1 to 100 rad/s. Young's modulus was determined with uniaxial unconfined compression at a rate of 10 mm/min.

To understand how sensory neurons interact with polyimide threads, an *in vitro* trial was performed. Dorsal root ganglia (DRG) were isolated from P1-P3 neonatal rats, trimmed, and cultured in 3D hydrogels in close proximity to "dummy" threads (i.e., no electrodes) for 1 week. The DRGs were then stained and neurite extension was quantified both towards and away from the polyimide thread.

To form complete TEENI devices for *in vivo* trials, a series of molds are used to hold the threads in place for hydrogel crosslinking (Figure 1). Tygon tube is wrapped around the threads and hydrogel solution is pipetted in. Hydrogels are crosslinked as described above. The tube is removed, and decellularized small intestinal submucosa (SIS) is placed under the crosslinked hydrogel/threads and sutured to form a tube for implantation.

A 6 week preliminary trial with "dummy" threads was performed on rat sciatic nerve (n=2). Devices had one of the two hydrogels and either no threads, 4 threads, or 10 threads. Briefly, 4 mm of rat sciatic nerve was transected and the device was implanted by suturing the SIS to the proximal and distal nerve stumps with a resulting gap of 5 mm. After 6 weeks rats were euthanized and histology was performed to assess axonal regeneration along the length of the device.

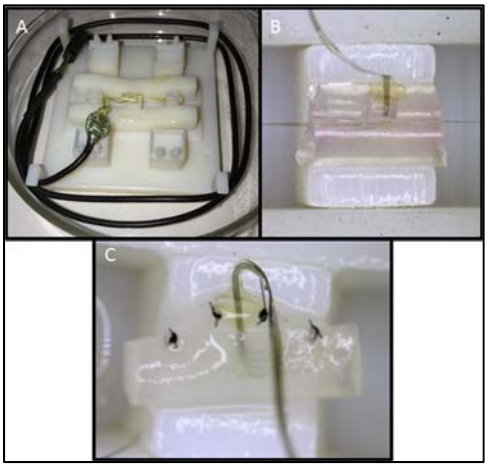

Figure 1. Fabrication of a TEENI device. A) Molds used to hold threads in place during fabrication. B) Hydrogel pipetted into Tygon tube wrapped around threads. C) SIS sutured around hydrogel and threads for implantation.

Results: Rheological testing showed high concentration MAHA hydrogels had the highest shear storage modulus at 106.8 ± 29.2 Pa, while peripheral nerve had the lowest at 23.2 ± 4.7 Pa. These values indicate that the hydrogels' mechanical properties are within the same order of magnitude as that of native peripheral nerve.

The Young's moduli of low MAHA and peripheral nerve was determined to be 0.212 ± 0.028 MPa and 3.318 ± 0.669 MPa, respectively. As with the storage modulus, these results indicate that the hydrogels are within the same order of magnitude as that of native peripheral nerve.

Preliminary results from the *in vitro* DRG trial indicate that the hydrogels support neurite extension in the presence of dummy threads.

After the 6 week preliminary *in vivo* trial, axons had regenerated from the proximal to distal end in all devices. Axon counts following the 6 week trial at both the proximal and mid-device sections indicate there was no significant difference between groups.

Conclusions: We have fabricated natural-based hydrogel scaffolds with mechanical properties mimicking native peripheral nerve tissue. Our hydrogel scaffold is able to hold thread electrodes stable during the fabrication and implantation procedures, and enable axonal regeneration *in vivo*.

References:

1. Ziegler-Graham K. Arch Phys Med Rehabil, 2008:89:422-429.
2. Navarro X. J Peripher Nerv Syst, 2005:10:229-258.