**Compound Action Potential Propagation in Microengineered Peripheral Neural Tissues**

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**Statement of Purpose:** The concept of using 3D cultures and microscale engineered tissues as benchtop models for toxicity screening and drug discovery has rapidly been gaining ground in recent years. This concept has been exploited fairly successfully for epithelial and metabolic tissues, where metabolites and other soluble analytes constitute the measurable read-outs. For peripheral neural tissue, where bioelectrical conduction over long distances may arguably be the most appropriate physiological read-out, application of engineered tissue for testing has been lagging. Our objective was to develop an organogypic, micro-physiological tissue model that mimics the morphology of peripheral nerve tissue and that supports physiological measurements analogous to clinical nerve conduction tests.

**Methods:** Rat embryonic day 15 dorsal root ganglion (DRG) explants were cultured in micromolds of polyethylene diacrylate (PEG), which was micropatterned with a dynamic mask projection lithography method described previously.[1] Neural constructs were incubated for up to two weeks, and neurite outgrowth was confined to narrow tracts filled with Puramatrix™, measuring ~400 µm in diameter, ~400 µm thick, and up to ~5 mm in length. Constructs were placed on an interface chamber perfused with bicarbonate-buffered artificial cerebral spinal fluid, and recordings were made using extracellular field potential electrodes. Recording electrodes were placed near cell somata in the vicinity of each ganglion, and constructs were stimulated with a bi-polar electrode at varying distances away from the ganglion along neurite tracts. Treatment with 0.5 µM tetrodotoxin (TTX) was used as a complete blockade of voltage-gated $Na^+$ channel activation, while 20 µM of 6,7-dinitroquinoxaline-2,3-dione + 50 µM of (2R)-amino-5-phosphonopentanoate (DNQX/APV) was used to block synaptic activity. Whole-cell patch clamp recordings were also taken after 7 days in culture. Tissue constructs were fixed in 4% paraformaldehyde, stained for β3-tubulin to identify neurites and DAPI for cell nuclei, and imaged with wide-field fluorescence and confocal microscopy. Some constructs were post-fixed in 1% $OsO_4$ and 2% uranyl acetate, dehydrated, embedded in Spurr resin, and cut into 80 – 100 µm sections before staining with uranyl acetate and lead citrate for TEM imaging.

**Results:** Wide-field fluorescence, confocal, and TEM imaging reveaed microengineered neural fiber tracts growing within the 3D volume of the Puramatrix hydrogel. The 3D constructs displayed relatively high degrees of parallel fiber growth, high density, and fasciculation. Most of the neurites are relatively small, on the order of ~1 µm in diameter, and though Schwann cells are seen to migrate along with the outgrowing neurites, there is little evidence of myelination.

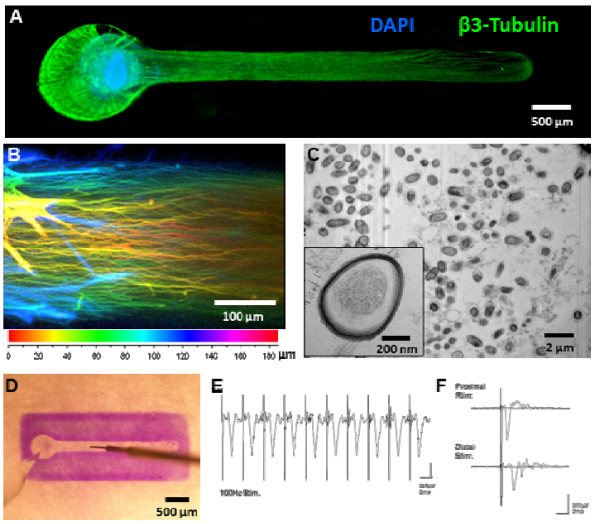


**Figure 1:** Morphology and physiology of micro-engineered neural fiber tracts. **A)** Fluorescence image of entire construct, stained for β3-tubulin and DAPI. **B)** Confocal z-stack of β3-tubulin stained neurites with depth color map. **C)** TEM cross-section of neural fiber tract, with single neurite shown in inset. **D)** Electrode placement for field recording. **E)** Action potentials recorded from 100-Hz stimulation pulse train. **F)** Compound action potentials recorded from proximal (top) and distal (bottom) stimulation locations. Scale = 500 µV / 5 msec.

Field potential recordings show population responses with consistent delay, amplitude, and envelope, even under high frequency (100 Hz) stimulation. Stimulation from a more distal location resulted in the expected delay of onset of the population spike. Application of TTX resulted in a complete and reversible blockade of responses, while application of DNQX/APV resulted in no change to the population responses. Single-cell intracellular recordings showed electrically-evoked responses whose rise times were independent of baseline voltage. These data strongly suggest that the observed population spikes are wholly compound action potentials propagating down the microengineered neural fiber tract.

**Conclusions:** Our data suggest that we can microengineer neural fiber tracts that resemble the morphology, physiology, and pharmacological responses of peripheral sensory nerve tissue. Results demonstrate the feasibility of developing benchtop physiological models for drug testing and discovery. Development of a model that is truly analogous to clinical physiological assessments, however, will likely require tissue constructs that are myelinated, with large fiber diameters, and that are amenable to more rapid physiological assessments.

[1]**Reference:** Curley, *J Biomed Mater Res* 2011; 99:532.
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