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Synthesis and Two-Photon Polymerization of a Cytocompatibility Tyramine Functionalized Hyaluronic Acid Hydrogel That Mimics the Chemical, Mechanical, and Structural Characteristics of Spinal Cord Tissue

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Abstract : Regeneration of the spinal cord after injury remains a great challenge due to the complexity of this organ. Inflammation and gliosis at the injury site hinder the outgrowth of axons and hence prevent synaptic reconnection and reinnervation. Hyaluronic acid (HA) is the main component of the spinal cord extracellular matrix and plays a vital role in cell proliferation and axonal guidance. In this study, we have synthesized and characterized a photo-cross-linkable HA-tyramine (tyr) hydrogel from a chemical, mechanical, electrical, biological and structural perspective. From our experimentation, we have found that HA-tyr can be synthesized with controllable degrees of tyramine substitution using click chemistry. The complex modulus (G*) of HA-tyr can be tuned to mimic the mechanical properties of the native spinal cord via optimization of the photo-initiator concentration and UV exposure. We have examined the degree of tyramine-tyramine covalent bonding (polymerization) as a function of UV exposure and photo-initiator use via Photo and Nuclear magnetic resonance spectroscopy. Both swelling and enzymatic degradation assays were conducted to examine the resilience of our 3D printed hydrogel constructs in-vitro. Using a femtosecond 780nm laser, the two-photon polymerization of HA-tyr hydrogel in the presence of riboflavin photoinitiator was optimized. A laser power of 50mW and scan speed of 30,000 µm/s produced high-resolution spatial patterning within the hydrogel with sustained mechanical integrity. Using dorsal root ganglion explants, the cytocompatibility of photo-crosslinked HA-tyr was assessed. Using potentiometry, the electrical conductivity of photo-crosslinked HA-tyr was assessed and compared to that of native spinal cord tissue as a function of frequency. In conclusion, we have developed a biocompatible hydrogel that can be used for photolithographic 3D printing to fabricate tissue engineered constructs for neural tissue regeneration applications.

Keywords : 3D printing, hyaluronic acid, photolithography, spinal cord injury
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