A Proposed Nanocomposite Hydrogel for Promoting Reinnervation Following Volumetric Muscle Loss

SARA PEPER, KAMAL AWAD, & NEELAM AHUJA, THY VO, MARCO BROTTO, & VENU VARANASI

Bone-Muscle Research Center, Musculoskeletal Nanobiomaterials Laboratory; College of Nursing & Health Innovation; University of Texas at Arlington; Arlington, TX

Category: Masters

Advisor / Mentor: Varanasi, Venu (venu.varanasi@uta.edu)

ABSTRACT

Skeletal muscle possesses an innate capacity for regeneration following damage, however, severe injuries resulting in significant tissue loss, categorized as volumetric muscle loss (VML) can overwhelm endogenous repair mechanisms. Recent evidence suggests that nanocomposite silica-based hydrogels (NC gels) possess rheological properties necessary for precise 3D bioprinting and promote secretion of endogenous neurotrophic factors for tissue repair. A shear behavior index between 0.2-0.6 is indicative of shear thinning behavior and describes the ability of a bioink to flow under a certain amount of shear stress. Bioinks displaying shear thinning behavior have pseudoplasticity, an essential property of muscle viscoelasticity, and are optimal for printing constructs with highly precise micro-architecture. **PURPOSE**: To determine the effect of ionic silicon (Si^{4+}) on neurotrophic factor release in C2C12 murine myoblasts and determine the effects of modified silica nanoparticles (SiOx-np) on the rheological properties of an established Gelatin Methacrylate-Sodium Alginate (GelMA-Alg) hydrogel for applications in skeletal muscle tissue engineering. **METHODS**: To determine the effects of Si4+ on neurotrophic signaling, C2C12 murine myoblasts were differentiated for 3 days in media containing 0.1mM, 0.5mM, and 1.0mM Si4+ and a silicon free control. An Enzyme-limited Immunosorbent Assay (ELISA) was performed to determine the level of the neurotrophic factor NRTN, a myokine released from skeletal muscle that enhances motor neuron recruitment and neuromuscular junction remodeling. The data was normalized to standard curves and origin was used to analyze the results. To determine the effects of SiOx-np on the rheological properties of GelMA-Alg, a 10% GelMA-2% Alg bioink was fabricated along with a 10% GelMA-2%Alg-2% SiO_x-np (0.6mM Si^{4+}) bioink. Flow curve tests were performed using an MCR702 multidrive rheometer from Anton Paar at 26°C and shear rate of 0.001-500s^{-1}. The results were analyzed based on a power law regression model using RheoCompass to determine the shear behavior index and the effects of SiOx-np on printability of the bioink. **RESULTS**: Following 3 days of differentiation it was determined that there was a significant increase in neurotrophic factor release in the 0.1mM group (1.20±0.04ng/mL, $p<0.05$), 0.5mM group (1.42±0.07ng/mL, $p<0.001$), and 1.0mM group (1.28±0.05ng/mL, $p<0.01$) compared to the silicon free control (1.14±0.06ng/mL). The shear behavior index was found to be similar between GelMA-Alg (0.3887) and GelMA-Alg-SiOx-np (0.37434) suggesting that the bioink displayed shear thinning behavior for precise 3D printing. **CONCLUSION**: The results of this study indicate that neurotrophic factor release in C2C12 myoblasts has an apical dose response to Si^{4+} *in vitro*. The shear thinning behavior of GelMA-Alg-SiOx-np is indicative of the pseudoplasticity of the material, suggesting constructs can be bioprinted with precise micro-architecture similar to skeletal muscle. These results suggest that GelMA-Alg-SiO_x-np constructs may provide a way to promote reinnervation following VML.