Biofunctional Hydrogels for Skeletal Muscle Constructs

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Statement of Purpose: A contractile muscle unit would play a significant role in ameliorating impaired morphologic and physiological function due to trauma, degenerative disease, aging or inactivity. Current therapeutics are limited to skeletal muscle grafts, as *in-vitro* engineered tissues are incapable of recapitulating natural muscle bundle architecture and function. Previous work involved 2D muscle strips, with naturally-occurring hydrogels, and incomplete examination of the effects of the scaffold on myogenic differentiation in a controllable manner. In this project, synthetic hydrogel scaffolds encapsulating C2C12 mouse skeletal muscle cells have been developed *in vitro* as a step towards regenerative medicine therapies for the enhancement or inducement of *de novo* functional skeletal muscle formation. The goal of this study was to identify key properties in functionalized poly(ethylene glycol) (PEG)-maleimide hydrogels that promote cell attachment, proliferation and differentiation for the development of multinucleated myotubes and functional skeletal muscle tissue constructs.

Methods: PEG-maleimide (PEG-MAL) 4-arm macromers were prefunctionalized with RGD adhesion peptide and cross-linked into a hydrogel by addition of cysteine-flanked MMP-degradable peptide sequences in the presence of C2C12 cell suspension. Fractional viability was assessed with samples stained fluorescently for Live/Dead at days 1, 3, 7. Extent of differentiation was analyzed based on cells stained positive for sarcomeric myosin after four days in differentiation media. Results were quantified using image analysis techniques. Contractility was demonstrated using Tyrode's solution. Inducement of alignment is being explored using microfabricated topographical cues and floating fascicle construct setup.

Results: The engineered bio-functionalized PEG matrix with maleimide cross-linking reaction chemistry gels rapidly with high cytocompatibility for ease of *in situ* delivery while still allowing "plug-and-play" design variation [1]. Significant differences in myoblast viability were observed as a function of cell seeding density, polymer weight percentage, and bioadhesive ligands. Optimized conditions for cell survival, required for myotube development, were carried over for differentiation assays (**Fig.1A**). The effects of incubation period in growth media and serum concentration in differentiation media on the extent of myotube development were studied (**Fig.1B,C**). 5%w/v PEG hydrogels functionalized with RGD peptides and cross-linked with protease-cleavable peptides incubated for 3 days before supplementation with 2% horse serum significantly increased expression of differentiated skeletal muscle protein and extent of multinucleation. Functionality was demonstrated by the decrease in extruded length of the hydrogel when stimulated with a contractile agent compared to a saline control (**Fig.1D**). Alignment has been observed on topographical cues and the fascicle construct method is being further explored (**Fig.1E**).

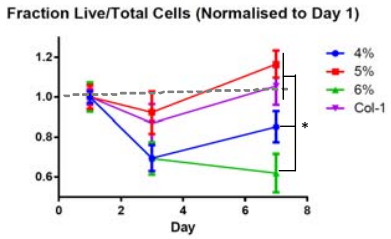
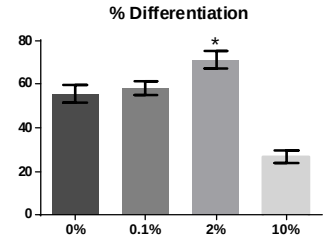
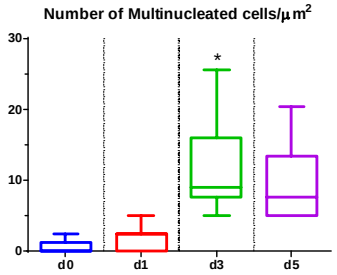


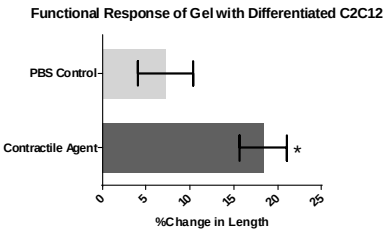
Fig.1.A. *Fraction of cells alive shows differences between 5% gels compared to 4% and 6% gels but not collagen type-I at day 7.*

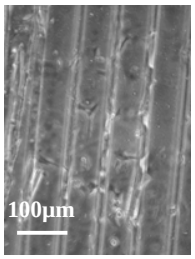


B. *% Differentiation is quantified as cells positive for differentiated marker out of total nuclei. More than 75% cells were differentiated in 2% HS, higher than other samples.*



C. *Analysis of extent of multi-nucleation. Differences found between 3 day incubation in growth media prior to differentiation vs. other groups with regards to both metrics.*





(D, left) *Change in length due to contraction in the presence if a PBS control and contractile agent, within 5 minutes.* ***(E, right)*** *C2C12 alignment within hydrogel as directed by micropattern.*

Conclusions: This work describes techniques to engineer a 3D microenvironment using synthetic hydrogels to promote the development of differentiated muscle tissue from skeletal muscle progenitor cells to form contractile units. These results show that synthetic hydrogel properties can be tuned to study the biophysical requirements of a cell from its environment, demonstrating the potential of engineered bio-functionalized PEG matrices for muscle regeneration.

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References:

1. Phelps EA, *Adv Mat.* 2012, 24(1):64-70