

Tunable Semi-synthetic Hyaluronic Acid Hydrogels Promote Muscle Regeneration After Rotator Cuff Injury

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INTRODUCTION: Skeletal muscle possesses a remarkable innate regenerative capacity after injury up to a critical sized defect. Tissue damage or loss beyond this critical threshold, known as volumetric muscle loss (VML), cannot be completely regenerated endogenously. Fibro-adipogenic progenitor cells (FAPs) have been identified as key regulators of skeletal muscle regeneration, expanding and adopting a brown-adipose tissue (BAT) phenotype to support muscle satellite cell (MuSC) myogenesis.^[1] We have developed tunable semi-synthetic hyaluronic acid (HyA) hydrogels for tissue regeneration and stem cell transplantation, allowing us to explore a range of parameters to support muscle regeneration including modulus, degradation rate, cell specificity, and growth factor sequestration.^[2-5] We hypothesize that our HyA hydrogel can promote human FAP (hFAP)-BAT differentiation, thereby inducing muscle regeneration. Having already demonstrated upregulation of the BAT-specific marker UCP1 after 7 days of hFAP culture within our HyA hydrogel, we sought to optimize our system using a variety of cell adhesion motifs specific to hFAP integrin binding.

METHODS: HyA hydrogels were synthesized according to previously reported protocols.^{[2][3]} Briefly, an HyA derivative carrying hydrazide groups (HyA-ADH) was synthesized, and N-acryloxysuccinimide was reacted with the HyA-ADH solution to generate acrylate groups on the HyA (AcHyA). For growth factor sequestration, SH-heparin was also synthesized to bind AcHyA via Michael-type addition. hFAPs were harvested from deltoid muscle biopsies (10 mg samples) performed on patients undergoing arthroscopic rotator cuff repair. Cells were encapsulated by mixing the cells into the macromer precursor solution containing one of four cell binding peptides (**Fig. 2A** - bsp-RGD(15), C16, Ag73, and P3). Gelation via Michael-type addition was initiated by addition of a bis-cysteine terminated peptide-cleavable crosslinker of varying Michaelis-Menten kinetics. BAT phenotype was assessed by immunostaining for UCP-1 and α SMA after 1, 3 and 7 days. ELISA for IL-10 was performed on the media at 1 and 7 day timepoints. For *in vivo* experiments, ten 9-month-old NSG mice underwent unilateral right suprascapular nerve denervation and combined supraspinatus and infraspinatus tendon transection, described previously.^[6] The mice were then randomized to treatment with 10 μ L saline (PBS) (n=5) or 10 μ L HyA-hydrogel (n=5). Mice were humanely sacrificed at 6 weeks and bilateral supraspinatus muscles were harvested and analyzed using immunohistochemistry.

RESULTS: Conjugation of different integrin engaging peptides impacted spreading and protein expression of FAPs after 7 days in culture as indicated by immunostaining (**Fig 1A**). Quantitative immunostaining analysis demonstrated increased expression of UCP1 in the HyA-bsp-RGD(15), C16, and P3 peptide hydrogels compared to HyA-T1 (**Fig. 1B**). By measuring promyogenic cytokine secretion in the media of hFAPs encapsulated within HyA-bsp-RGD(15) hydrogels we found that IL-10 secretion was significantly increased after 7 days (**Fig 1C**). *In vivo*, HyA-bsp-RGD(15) hydrogel implantation in a rotator cuff injury model resulted in a significant decrease in relative muscle weight loss compared to the PBS control (**Fig 1D**). Trichrome staining showed fibrosis was also significantly decreased in the HyA-bsp-RGD(15) hydrogel group compared to PBS (**Fig 1E**). Furthermore, fatty infiltration was reduced to the level of the control group in the HyA-bsp-RGD(15) hydrogel treatment group compared to PBS. To measure vascularization, laminin and CD31 expression were quantified via immunostaining, revealing a significant increase of both markers in the HyA-bsp-RGD(15) hydrogel group compared to PBS treatment.

DISCUSSION: Our *in vitro* work demonstrates the ability of our HyA hydrogels to support BAT differentiation of hFAPs as well as to increase promyogenic cytokine secretion over time. *In vivo*, the HyA-bsp-RGD(15) hydrogel reduces fibrosis, fatty infiltration, and muscle atrophy while supporting vascularization of injured muscle tissue, and ultimately muscle regeneration. Together, our results suggest that hydrogel-driven FAP-BAT differentiation may contribute to the positive regenerative outcomes after treatment of muscular injury with our hydrogel.

SIGNIFICANCE & CLINICAL

RELEVANCE: The materials developed during these studies provide the foundation for both *in situ* and *in vivo* biomaterial therapy approaches for treatment of VML in the clinic. Furthermore, understanding the biophysical and material parameters that influence FAP-BAT differentiation enable their translation into therapeutics for muscle regeneration.

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