Enhanced satellite cell differentiation and muscle regeneration by semi-synthetic Hyaluronic acid hydrogel-mediated delivery of Fibro-Adipogenic Progenitors in Volumetric Muscle Loss

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Introduction: Volumetric muscle loss (VML) injuries are characterized by a degree of tissue loss that exceeds the endogenous capacity of muscle to regenerate, resulting in permanent functional and structural deficits. These injuries result from trauma, as well as a host of congenital and acquired diseases, with limited treatment options. Thus, there is a clear need for solutions that allow for functional and structural muscle repair. Fibro-Adipogenic Progenitors (FAPs) are a group of muscle residential progenitor cells, characterized by their cell surface marker platelet-derive grow factor alpha (PDGFRα), and are thought to play an important role in muscle regeneration by adopting a brown-adipose tissue (BAT) phenotype and supporting satellite cell (SCs) myogenesis [1]. We have developed semi-synthetic hyaluronic acid (HyA) hydrogels for tissue regeneration and stem cell transplantation, wherein we tune a range of parameters including modulus, degradation rate and growth factor sequestration. In this study, we assessed the capacity of our semi-synthetic HyA hydrogel to deliver FAPs, promote myogenesis of SCs, and promote muscle regenerate in a pre-clinical model of VML injury.

Methods: HyA-based hydrogels were synthesized as previously described [2-3]. Briefly, HyA derivative carrying hydrazide groups was synthesized and reacted with acryloxysuccinimide to generate acrylate groups on the HyA (AcHyA). Separately, thiolated heparin (Heparin-SH) was synthesized, along with an AcHyA derivative containing a 15 amino acid RGD cell binding motif (AcHyA-RGD) [4]. SCs were harvested from healthy 3 month old C57B/L6 mice and FAPs were harvested from 3 month old UCP1-tdTomato Reporter mice with CD31-CD45-ITGA7-SCA1+ using BD Aria III flow cytometry. For *in vitro* studies, FAPs were cultured in HyA hydrogels alone or in co-culture with SCs to assess their capacity to support SCs myogenesis. Cells were mixed with HyA hydrogel precursors and gelation initiated by addition of a bis-cysteine terminated peptide-cleavable crosslinker (MMP13) as previously described [2-3]. UCP-1 expression in FAPs was assessed by measuring expression of the tdTomato UCP-1 reporter. Myogenesis of SCs was examined by staining of myosin heavy chain. *In vivo*, VML injury of 4 mm diameter was created in the proximal one third of the tibialis anterior (TA) muscle and FAPs encapsulated and delivered within the HyA hydrogel. The TA muscles were harvested after 3 weeks and muscle weight loss and cross-sectional area (CSA) of myofibers were measured. For both *in vitro* and *in vivo* studies Matrigel served as a control hydrogel.

Results: UCP-1 expression by FAPs encapsulated alone in HyA hydrogels or Matrigel was upregulated two-fold compared with 2D culture, indicating differentiation of FAPs toward a BAT-like phenotype. When co-cultured with SCs in the HyA hydrogel, there was an increase in the percentage of UCP1(+) FAPs to 45.38±2.55% after 7 days, compared to 23.37± 4.98 in Matrigel. Myotube fusion index of SCs was also significantly higher in the HyA hydrogel co-culture group (57.43±3.36%) compared to that in Matrigel (34.32±4.12%), and the 2D co-culture control group (12.4±2.47). *In vivo*, delivery of FAPs via HyA hydrogel showed the lowest TA muscle weight loss in the injured muscle after VML injury compared to FAPs delivered in Matrigel or saline groups **(Figure 1)**.

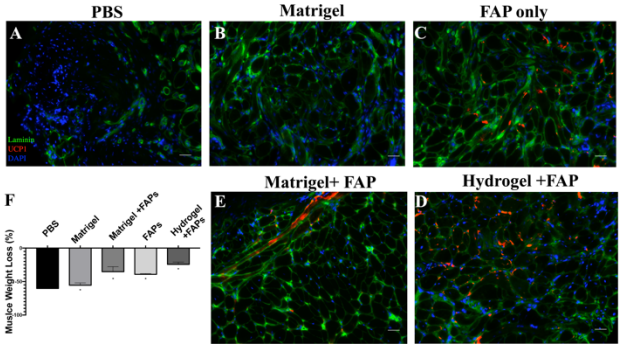

Figure 1: HyA hydrogel delivery of FAPS promotes recovery of TA following VML injury. Immunofluorescent staining of TA muscles following VML injury treated with (A) PBS, (B) Matrigel, (C) FAPs, (D) HyA hydrogel with FAPS and (E) Matrigel with FAPs. (F) Quantification of TA muscle weight loss in vivo after 3 weeks. [Green = Laminin, Red = UCP-1, Blue = DAPI]

Conclusions: HyA-based hydrogels supports BAT-like differentiation of FAPs, and subsequent myogenesis of SCs in a co-culture system. *In vivo* data demonstrates the potential of the HyA-based hydrogel system to deliver FAPs and promote regeneration and recovery in a preclinical model of VML injury. This HyA-based hydrogel has previously demonstrated a potential to support BAT-like differentiation of progenitor cells [5], and this mechanism along with the sequestration of pro-myogenic factors via the inclusion of heparin, most likely mediates its regenerative capability.

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