Biomolecule Delivery to Synergistically Mobilize and Locally Recruit Bone Marrow Cells Enhances Muscle Regeneration Following Rotator Cuff Tear

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Statement of Purpose: Rotator cuff tear affects approximately 20% of the general population and results in significant muscle degeneration, which correlates with increased re-tear rates after surgical repair.[1,2] Pro-regenerative cell recruitment to injured muscle may be a method to promote increased muscle healing.

Localized and sustained delivery of stromal cell-derived factor-1α (SDF-1α), a chemoattractant protein, to injured muscle via degradable heparin-based microparticles (MPs) has been shown to recruit pro-healing cells to injured muscle after rotator cuff injury.[3] Mobilizing pro-healing cells that are responsive to SDF-1α with VPC01091(VPC)[4,5], a bone marrow mobilizing agent, could potentially enhance the endogenous cell recruitment previously observed. It is therefore hypothesized that this method to synergistically "push" pro-healing cells into circulation and "pull" them to sites of injury would increase the number of pro-regenerative cells in the supraspinatus muscle following rotator cuff injury, and promote muscle regeneration, more than either treatment alone.

Methods: Degradable MPs were fabricated via water-and-oil emulsion, followed by free-radical polymerization and Michael type addition between 10 wt% Hep^{-N} methacrylamide, 90 wt% poly (ethylene glycol) diacrylate, and 35 mM dithiothreitol (Sigma). To load SDF-1α onto MPs, 1.0-1.2 μg sterile human SDF-1α (R&D Systems) was added to 0.6 mg MPs. VPC (Avanti Polar Lipids) was dissolved at 1 mg/mL in 2% 2-hydroxypropyl-B-cyclyodextrin H_2O solution. Rotator cuff injury was induced in male, Sprague Dawley rats via unilateral transection of the supraspinatus and infraspinatus tendons and denervation of the suprascapular nerve. Immediately following rotator cuff injury, VPC was administered via I.P. injection at either 1 mg or 5 mg/kg animal weight, and SDF-1α loaded MPs were injected into the supraspinatus muscle.

7 days following injury and treatment, supraspinatus muscles were harvested, embedded in OCT and cryosectioned. Muscle cross sections were stained for embryonic myosin heavy chain (eMHC+) regenerating muscle fibers. Mounted slides were imaged using a Zeiss LSM 700 confocal microscope with a 10x objective to visualize eMHC, within outlined muscle fibers with cell nuclei. Muscles were also digested with collagenase IA (Sigma) and stained with antibodies to quantify myeloid cells (CD11b+), macrophages (CD11b+CD68+), M1-like, pro-inflammatory macrophages (CD11b+CD68+CD163-) and M2-like, anti-inflammatory macrophages (CD11b+CD68+CD163+), as well as mesenchymal stem cells (MSCs, CD29+CD44+CD90+) via flow cytometry (BD Biosciences FACS-AriaIIIU) at 3 and 7 days.

Results: 7 days following injury and treatment, significantly more eMHC+ muscle fibers were observed for VPC+SDF, VPC only and SDF only treatment groups, than in untreated injury muscles. However, significantly more eMHC+ fibers were observed in muscles treated with VPC+SDF-1α than SDF-1α treatment.

After 3 days, significantly more M2-like and M1-like macrophages were observed with VPC+SDF-1α and VPC treatment than injury. M2:M1 macrophage ratios were significantly higher in VPC+SDF and VPC groups than SDF-1α and injury. By day 7, significantly more M2-like macrophages were observed in muscle for all treatments, compared to injury. Significant differences in M1-like macrophages were observed between SDF-1α and VPC treatments. Only VPC+SDF-1α and VPC treatments significantly increased the M2:M1 ratio.

After 3 days, significantly more MSCs were observed in muscle with VPC+SDF-1α and VPC treatment than all groups. By day 7, all treatments resulted in significantly more MSCs than injury.

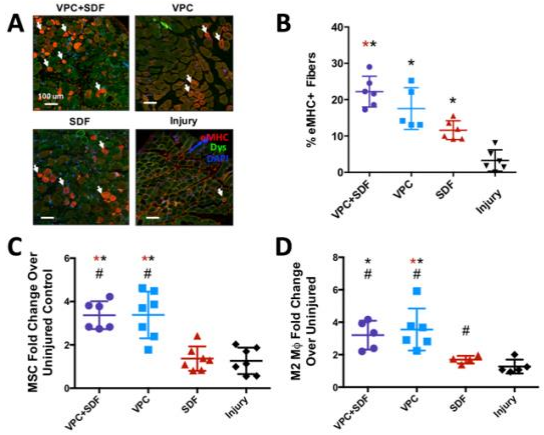

Figure 1. Immunohistochemistry staining indicates eMHC+ regenerating muscle in VPC+SDF treated muscles 7 days post injury. (A) Representative images of muscle cross-sections stained with eMHC (red), dystrophin (green), and DAPI (blue). White arrows indicate example eMHC+ muscle fibers, scale bars are 100 μm; (B) Quantification of eMHC images; $p \leq 0.05$; n = 5-6. Fold change of (C) MSCs and (D) M2-like macrophages recruited to supraspinatus muscle compared to uninjured contralateral controls 3 days post injury and treatment. Significantly different than: #uninjured; * injury; **SDF and injury; $p \leq 0.05$; n = 4-7.

Conclusions: Codelivery of VPC and SDF-1α or VPC alone increases the number of MSCs and myeloid cells in injured muscle at an earlier time point than SDF-1α alone, suggesting that bone marrow mobilization may hasten recruitment of pro-healing cells. Overall, the ability of codelivery of VPC & SDF-1α to alter the early cellular milieu, and further increase the eMHC+ regenerating fiber quantity over that observed in muscle treated with SDF-1α alone, indicates that this "push-pull" method may be a promising means to promote muscle regeneration after rotator cuff injury.

References: [1]Yamamoto AJ. Shoulder Elbow Surg. 2010. [2]Gladstone JN. Am J Sports Med. 2007. [3]Tellier LE. Regen. Eng. Transl. Med . 2018. [4]Ogle ME. Stem Cells. 2016. [5]Selma JM. Cell. Mol. Bioeng. 2018.