Hydrogel Surfaces Promote MSC Expansion and Proregenerative Priming for Rotator Cuff Muscle Healing

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Statement of Purpose: Mesenchymal stem cells (MSCs), through their paracrine immunomodulatory and regenerative activity, have therapeutic potential for inflammatory and degenerative diseases such as muscle degeneration after severe rotator cuff tear. To achieve therapeutically relevant cell numbers, MSCs must undergo culture expansion prior to delivery. Novel biomaterial culture surfaces present an opportunity to pre-condition cells to improve therapeutic attributes including pro-regenerative secretome and maximize cell expansion by reducing replicative senescence. The objective of this study was to screen several hydrogel formulations, with varied mechanical properties and bioactive components, for their ability to improve MSC secretome, proliferation, and to reduce senescence. We hypothesized that softer surfaces would facilitate cell-cell aggregation, which is associated with increased paracrine activity and lower proliferation, while stiffer gels would facilitate cell-gel interactions and sustain proliferation without senescence. We further investigated the application of MSCs expanded on hydrogel substrates for the treatment of muscle degeneration following severe rotator cuff tear in a rodent surgical model.

Methods: Human bone marrow-derived MSCs (RoosterBio) were cultured on hydrogel surfaces or tissue culture plastic (TCP) in DMEM (1g/L glucose) with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Inc.). Hydrogels were fabricated from combinations of poly(ethyleneglycol)-diacrylate (PEGDA), acryl-PEG-GRGDS (RGD, 1mM), methacrylamide-functionalized heparin (Hep), desulfated heparin (Hep-), or hyaluronic acid (HA), and cross-linked with free-radical initiators. Compressive modulus was either 30kPa or 100kPa. Secretome was analyzed by Luminex multiplex ELISA and cell number by PicoGreen DNA quantification. Senescence was assessed by beta-galactosidase (ß-gal) staining. Rotator cuff tear was modeled in male Sprague-Dawley rats by surgical resection of the supra- and infra-spinatus tendons and denervation of the suprascapular nerve (Tellier, LE. *Regen Eng and Transl Med* 2018, 4:92-103). MSCs (1x 10^6 cells) cultured on hydrogels were injected into the distal portion of the supraspinatus muscle at the time of injury. Muscles were cryosectioned and stained by standard immuno-histochemical methods.

Results: Hydrogel stiffness was the dominant determinant of secretory phenotype of MSCs. Softer gels of 30kPa compressive modulus elicited overall more paracrine secretion per cell compared to 100kPa modulus gels or >GPa TCP (Fig 1A-C). No increase of soluble factors was detected on Hep hydrogels, however cells on Hep- and HA gels produced a secretome similar to RGD gels. Soft 30kPa gels did not support expansion of cells, however, MSCs on 100kPa Hep gels expanded similar to TCP. To determine whether hydrogel culture reduced replicative senescence, MSCs were cultured for 3 passages on either TCP or 100kPa RGD, Hep, Hep-, or HA hydrogels. ß-gal staining indicated wide-spread senescence of TCP-cultured MSCs; however, all hydrogel surfaces abrogated senescence (Fig 1D). To assess the regenerative capacity of hydrogel-cultured MSCs in a disease model, MSCs were maintained on 100kPa RGD gels for 4 days prior to transplantation into the supraspinatus muscle following rotator cuff injury. After 7 days, human MSCs were identified by human nuclear antigen (hNu) staining in the muscle (Fig 1E). Regenerating fibers were identified nearby the location of MSCs by embryonic myosin heavy chain (eMHC) expression in serial sections (*, Fig 1F).

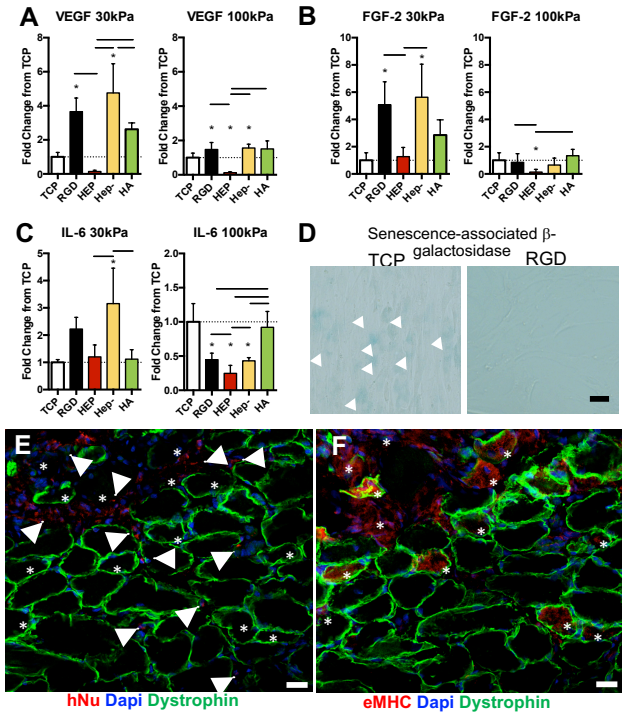

Figure 1. (A) VEGF, (B) FGF-2, and (C) IL-6 secretion from cultured MSCs. (D) Abrogation of senescence on 100kPa gels (arrows, ß -gal). (E-F). Serial sections of muscle 7 days post-injury and injection. (E) hNu+ MSCs (arrowheads, red) in muscle between dystrophin+ fibers (green). (F) Section showing regenerating eMHC+ muscle fibers (*, red) close to injected MSCs labeled in (E). (Scale bars 20um).

Conclusions: Hydrogel culture surface composition can be tuned to prime cells for paracrine activity (primarily 30kPa gels) or to support healthy expansion of MSCs (100kPa gels). All types of 100kPa surfaces decreased MSC senescence compared to TCP, thereby increasing cell health for *in vivo* delivery and increasing the expansion potential of MSCs *in vitro*. Localization of hydrogel-expanded MSCs in serial sections near the site of regenerating muscle fibers suggests that MSCs pre-cultured on 100kPa hydrogels promote regeneration *in vivo*.