Fibrochondrogenic differentiation of hMSCs on Nanofiber Scaffolds with and without Hydroxyapatite Nanoparticles

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Statement of Purpose: Rotator cuff tendon tears represent one of the most common shoulder injuries, with 75,000 repairs performed annually in the U.S[1]. The tendon inserts into bone through a fibrocartilagenous interface, with aligned non-calcified collagen fibers traversing the interface as well as calcified fibers penetrating into subchondral bone[3-5]. This multi-region interface is critical for minimizing stress concentrations and facilitating load transfer between tendon and bone. As full-thickness cuff tears often occur at the supraspinatus tendon-to-bone insertion[2], regeneration of this interface following surgical repair can promote long-term stability and restore the functionality of the rotator cuff. **Our approach** is to develop a biomimetic scaffold aimed at promoting the biological fixation of tendon to bone by facilitating the regeneration of the tendon-bone insertion. To this end, nanofiber-based poly(lactide-*co*-glycolide) (PLGA) scaffolds with and without a hydroxyapatite (HA) nanoparticle phase have been formed[6]. **The objective of this study** is to evaluate the fibrochondrogenic differentiation of human mesenchymal stem cells (hMSCs) on PLGA and PLGA-HA nanofiber scaffolds. It is hypothesized that hMSC response will be regulated by both mineral presence and biochemical stimulation.

Methods: *Scaffold Fabrication and Characterization*–Aligned nanofibrous scaffolds composed of PLGA (85:15, Lakeshore) & HA nanoparticles (100-150nm, Nanocerox) were produced via electrospinning[6,7]. PLGA nanofiber scaffolds with 0% and 5% HA (w/w) were formed. The scaffolds were examined by SEM (n=3) and mineral presence was confirmed with EDAX analysis (n=3). Tensile mechanical properties (n=5) were also measured (Instron, 5 mm/min). *Cells & Cell Culture*–hMSCs (Cambrex) were seeded on the scaffolds ($3.14x10^4$ cells/cm^2) and cultured in ITS+ media with 0.1µM dexamethasone, 50µg/mL ascorbic acid[8,9]. For fibrochondrogenic differentiation, 10 ng/mL of TGF-β3 was added. *End-Point Analyses (at 1 ,3, 7, 14, 28 days)*–Cell viability and morphology (n=3) were examined by the Live/Dead assay, and proliferation (n=5) by PicoGreen assay. Glycosaminoglycan (GAG) deposition (n=5) was quantified by DMMB assay and collagen deposition (n=5) was determined by the Sircol assay.

Results: *Scaffold Characterization*-SEM revealed that the HA particles were well distributed and embedded within the PLGA nanofibers (Fig. 1A). Moreover, both Ca and P peak intensities increase with the addition of HA. While a decrease in yield strength was found with the addition of HA, no difference in elastic modulus was found between the 0 and 5% HA scaffolds (Fig. 1B).

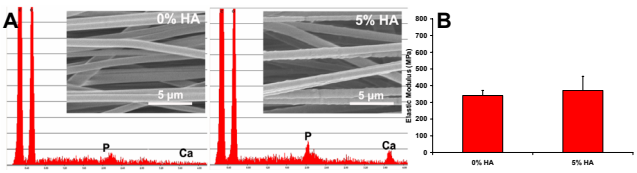

***Fig. 1**: **A)** SEM micrographs (4000x) and EDAX spectra of 0% and 5% HA nanofiber scaffolds, **B)** Yield strength of 0% and 5% HA scaffolds.*

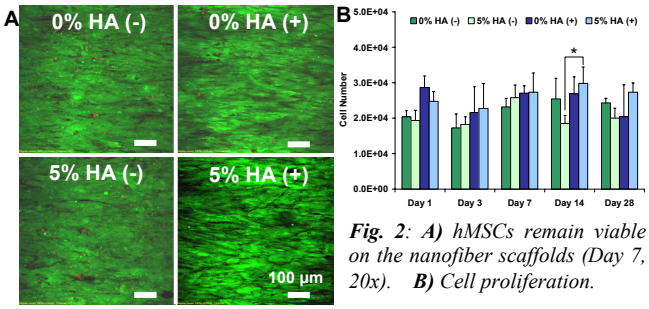

***Fig. 2**: **A)** hMSCs remain viable on the nanofiber scaffolds (Day 7, 20x). **B)** Cell proliferation.*

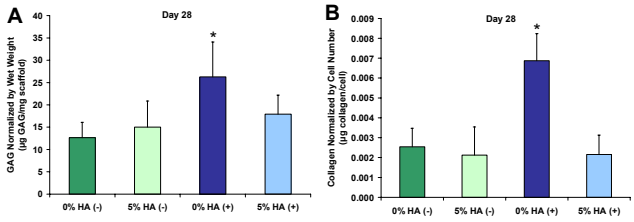

***Fig. 3**: Normalized GAG **(A)** and collagen **(B)** content at day 28.*

Fibrochondrogenic Differentiation-The hMSCs aligned along the nanofiber long axis and remained viable on all substrates (Fig. 2A), with minimum proliferation found in the serum-free ITS media. Stimulation by TGF-β3 led to a significant increase in cell number at day 14 for the 5% HA group. The normalized GAG and collagen content were significantly greater for the 0% HA supplemented group compared to all other groups (Fig. 3A,B).

Effects of Scaffold Mineral Phase-While no significant difference in biosynthesis was observed between the PLGA and PLGA-HA scaffolds, under TGF-β3 stimulation, the presence of HA nanoparticles inhibited proteoglycan and collagen production (Fig. 3, $p<0.05$).

Discussion and Conclusions: The results of this study demonstrate that both types of nanofiber scaffolds (PLGA, PLGA-HA) support hMSC viability and biosynthesis. Under TGF-β3 stimulation, the cells expressed both collagen I and II, while producing a matrix rich in GAG and collagen on the PLGA scaffold. Incorporation of HA nanoparticles into the PLGA nanofibers modulates matrix production by these cells in the presence of TGF-β3. These findings demonstrate that TGF-β3 promotes fibrochondrogenic differentiation of hMSCs on the nanofiber scaffolds, and moreover, cell response is regulated by the HA nanoparticles. Future studies will focus on optimizing the PLGA and PLGA-HA composite scaffolds for promoting hMSC-mediated rotator cuff repair and integration with subchondral bone.

References: [1]Vitale *et al*., 2007; [2]Iannotti *et al*., 1994; [3]Woo *et al*., 1987; [4]Benjamin and Evans, 1990; [5]Cooper and Misol, 1970; [6]Moffat *et al*., 2008; [7]Matthews *et al*., 2002; [8]Li *et al*., 2005; [9]Baker and Mauck, 2007.