Effect of Nanofiber Mineral Content on Human Mesenchymal Stem Cell Osteogenesis

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Statement of Purpose: Tissue engineering represents a promising approach to regenerate bone for the upwards of 1 million fractures annually in the US[1]. Our approach is to design a nanofiber-based scaffold for bone regeneration, as these scaffolds can directly mimic the collagenous bone matrix[2], in conjunction with multi-potent mesenchymal stem cells (MSC)[3]. It has been well established that the incorporation of ceramics, such as hydroxyapatite (HA), the primary mineral found in bone[4], in tissue engineered scaffolds can enhance osteogenesis[5,6]. However, previous studies have evaluated the effect of incorporating HA nanoparticles within nanofibers on MSC osteogenesis coupled with osteogenic induction factors[7,8]. **The objective of this study** is to evaluate the osteogenic differentiation of human MSC on composite polymer-ceramic nanofiber scaffolds and determine the optimal mineral content for osteogenic induction, in the absence of chemical factors. It is **hypothesized** that osteogenic differentiation will be enhanced with increasing mineral content.

Methods: *Scaffold Fabrication and Characterization:* Aligned nanofiber scaffolds composed of a 5:1 blend of poly(lactide-*co*-glycolide) (PLGA 85:15, Lakeshore) and poly(ε-caprolactone) (PCL) (Sigma) were fabricated with 0%, 15%, 25% and 35% (w/w) HA nanoparticles (100-150nm, Nanocerox) via electrospinning[9]. Scaffold morphology was examined by SEM (n=3), mineral presence assessed with FTIR analysis (n=3) and mineral content measured via TGA (n=5). *Cells & Cell Culture:* Human MSCs (Texas A&M Health Science Center) were seeded on scaffolds ($3x10^4$ cells/cm^2) and cell response was analyzed after 6 hours, 12 hours and 1, 7, 14 and 28 days. *End-Point Analyses:* Cell viability and morphology (n=2) were examined by the Live/Dead assay and proliferation (n=5) by the PicoGreen assay. Alkaline phosphatase (ALP) activity (n=5) was quantified using a colorimetric assay and collagen deposition (n=5) was determined by the hydroxyproline assay. Osteogenic differentiation (n=5) was assessed via real-time RT-PCR for collagen I, ALP, RUNX2, osteocalcin (OC) and osteopontin (OP). *Statistical Analysis:* ANOVA and the Tukey-Kramer post-hoc test used for all pair-wise comparisons (*p<.05).

Results: *Scaffold Characterization:* SEM revealed that the HA nanoparticles were well distributed and embedded within the nanofibers (Fig. 1). HA presence was confirmed via PO_4^{3-} absorption bands at 1031 cm^{-1}, 603 cm^{-1} and 564cm^{-1} (Fig. 2). TGA verified the mineral content incorporated into scaffolds (Fig. 2). *Cell Growth and Matrix Production:* No differences in cell number or collagen were observed after four weeks (Fig. 3). *Osteogenic Differentiation:* ALP activity was significantly higher on scaffolds containing 25% and 35% HA after 12 hours and remained elevated until day 7, as compared to the 0% and 15% HA groups (Fig. 4). Gene expression analysis after 28 days revealed the upregulation of ALP, RUNX2, and OC on 25% and 35% HA groups as compared to the 0% and 15% groups. OP was only upregulated on the 35% HA scaffolds (Fig. 4).

Discussion/Conclusions: In this study, osteogenic differentiation of human MSCs was assessed on composite polymer-ceramic scaffolds without concurrent growth factor stimulation. Differentiation only occurs on scaffolds containing 25% and 35% HA and OP is only upregulated on the 35% HA scaffolds, suggesting that greater mineral content enhances osteogenic differentiation. These findings may be due to the 35% HA scaffolds more closely mimicking the mineral content of bone (~60% HA[10]). Similarly, Peng *et al.* reported an upregulation of RUNX2 and ALP expression by murine MSC on an unaligned chitosan-HA nanofiber system containing 30% HA, without osteogenic medium. It was reported that these effects were further enhanced with the addition of growth factors[11]. However, Polini *et al.* reported the upregulation of RUNX2 on unaligned PCL nanofibers containing 36% tricalcium phosphate, without chemical factors, though not on PCL-HA fibers[12]. The results of this study indicate that nanofiber HA content alone can induce MSC osteogenesis. Future work will evaluate the mechanisms behind these findings and investigate the additive effect of chemical stimulation.

References: [1]Salgado *et al.*, 2004 [2]Wang *et al.*, 2010 [3]Pittenger *et al.*, 1999 [4]Boskey *et al.*, 1981 [5]Marra *et al.*, 1999 [6]Khan *et al.*, 2008 [7]Lee *et al.*, 2009 [8]Chen *et al.*, 2011 [9]Moffat *et al.*, 2009 [10]Osborn *et al.*, 1980 [11]Peng *et al.*, 2012 [12]Polini *et al.*, 2011

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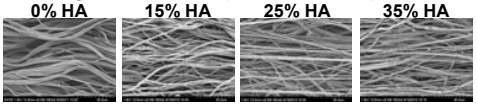

Fig 1. SEM of nanofiber scaffolds.

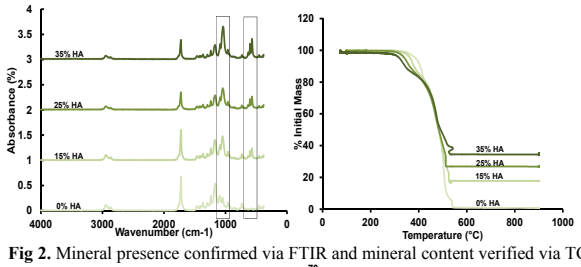

Fig 2. Mineral presence confirmed via FTIR and mineral content verified via TGA.

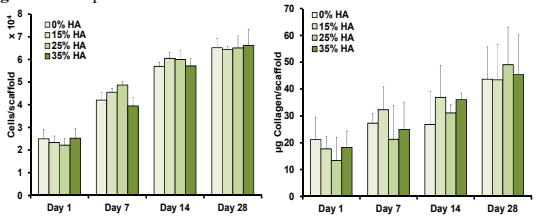

Fig 3. No significant difference in cell number or collagen was measured between groups.

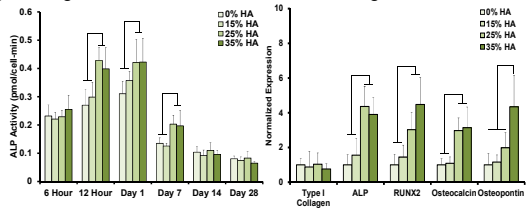

Fig 4. ALP activity was elevated on the 25% and 35% HA groups beginning at 12 hours. Several osteogenic markers were upregulated on the 25% and 35% HA scaffolds at day 28 though only OP was upregulated on the 35% HA scaffolds.