Composite Hydrogel-Hydroxyapatite Scaffold for Regeneration of the Cartilage-Bone Interface

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Introduction: Osteoarthritis or cartilage degeneration is the leading cause of physical disability among Americans. Articular cartilage integrates with bone via the osteochondral interface, a calcified cartilage region that separates the uncalcified deep zone cartilage from subchondral bone[2]. Regeneration of this critical interface would promote the integration of cartilage grafts[1]. We have designed a composite scaffold of hydroxyapatite (HA) and agarose for interface formation, as the HA phase resembles the mineral of the calcified cartilage region and agarose has been successfully utilized for cartilage tissue engineering[3,4]. Since both HA aggregates and hypertrophic chondrocytes are observed at the osteochondral interface[5], the **objectives of this study** are to examine the effects of **1)** HA particle size and **2)** stimulation with triiodothyronine (T3), a known promoter of hypertrophy[4] on deep zone chondrocyte (DZC) growth, biosynthesis and hypertrophy in the hydrogel+HA scaffold. **It is expected** that T3 stimulation will promote DZC hypertrophy and calcification, and cell response will be HA particle size-dependent.

Methods: DZC were isolated from the bottom 30% of neonatal calf cartilage tissue[8] and maintained in ITS media with 50 µg/mL ascorbic acid. Cells combined with 2% w/v nano-HA (Nanocerox, 100 nm) or micro-HA (Sigma, 20 um) were mixed with 4% agarose (10 million cells/ml). HA distribution within the hydrogel was confirmed by EDAX and FTIR (Fig. 1). *Experimental groups* were stimulated with 50 nM T3 for the first 3 days of culture. *Control groups* included DZC in T3-untreated, HA-free scaffolds and corresponding acellular scaffolds. Samples were analyzed on Days 1, 7, and 14 for total DNA (n=6), alkaline phosphatase activity (ALP,n=6), and production of glycosaminoglycans (GAG, n=6) and collagen (n=6) with corresponding histology (n=2). Additionally, gene expression (n=3) for collagen I, II, X, and MMP13 was determined by RT-PCR, with β-actin as housekeeping gene. Shear (n=3) and compressive moduli (n=3) were determined under static unconfined compression (0.025N tare load, 15% strain) and dynamic torsional shear using a rheometer (TA Instruments, 0.01 radians, 1 Hz)[5]. ANOVA and Tukey–Kramer tests were used for statistical analyses ($p<0.05$).

Results: A significant increase in cell number is observed by Day 7 for all groups. Culturing with micro-HA increases cell number, while T3 reduces cell number by Day 14 in the absence of HA. Extensive matrix deposition is observed over time for all groups, and significantly higher GAG and collagen deposition are found by Day 14 in the micro-HA group with T3 (Fig. 2). As expected, T3 enhances DZC ALP activity and the expression of hypertrophic markers, although both are suppressed at Day 7 with the addition of micro-HA (Fig. 3). Alizarin Red staining reveals the presence of scaffold-HA and cell-mediated mineralization. As expected, cell-seeded scaffolds exhibit higher shear mechanical properties than the acellular controls due to matrix elaboration. Higher shear modulus is found with 1.5% HA, and increasing micro-HA concentration from 1.5% to 3% resulted in higher ($p<0.05$) compressive properties (Fig. 4).

Discussion: These results demonstrate that T3 stimulates DZC hypertrophy and promotes calcification. Previous studies have shown that the presence of HA modulates DZC biosynthesis by suppressing ALP activity and increasing collagen deposition[6]. Our results suggest that these changes are also dependent on HA particle size, with the micro-HA stimulating the formation of a calcified matrix rich in GAG and collagen that resembles the calcified cartilage interface. Furthermore, mechanical properties of the composite scaffold is modulated by scaffold HA content. Future studies will focus on optimizing the scaffold for biosynthesis, followed by *in vivo* evaluation for interface regeneration.

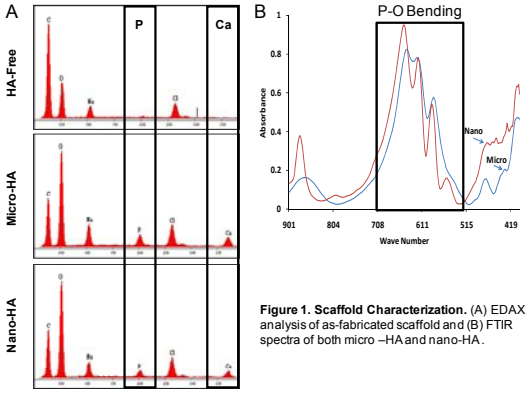

Figure 1. Scaffold Characterization. (A) EDAX analysis of as-fabricated scaffold and (B) FTIR spectra of both micro –HA and nano-HA.

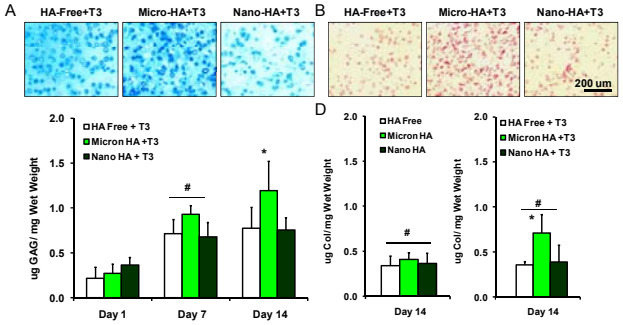

Figure 2. Matrix Deposition. (A) Quantiative GAG with corresponding Alcian Blue staining for T3-treated groups and (B) quantiative collagen with corresponding Picrosirius Red (10x, day 14; *p<0.05 between groups, #: p<0.05 over time).

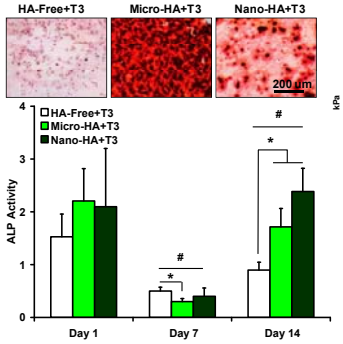

Figure 3. Mineralization. ALP activity of T3-treated groups with corresponding Alizarin Red staining (10x, day 7, *: p<0.05 between groups, #: p<0.05 over time).

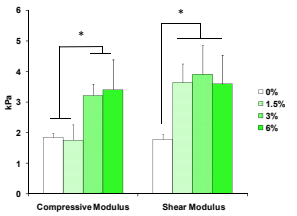

Figure 4. Mechanical Properties. Compressive and shear mechanical properties on Day 1 for samples with 0%-6% micro-HA (*p<0.05).

REFERENCES: *1) Hunziker et al, 2001. 2} Bullough et al, 1993. 3) Mauck et al.,2006. 4)Cook et al, 1993. 5) Arsenault et al, 1988. 6) Rosenthal et al., 1993. 7) Zhu et al, 1993.8) Khanarian et al, 2007. 8} Jiang et al, 2008.*
ACKNOWLEDGMENT: *NIH-NIAMS 5R01AR055280-02*