Composite Hydrogel-Ceramic Scaffold Design for Regeneration of the Cartilage-Bone Interface

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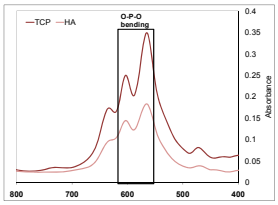
Statement of Purpose: Osteoarthritis results in cartilage degeneration and is the leading cause of physical disability among Americans. Articular cartilage integrates with bone via the osteochondral interface, a calcified cartilage region that separates the uncalcified deep zone cartilage from subchondral bone[1]. Regeneration of this critical interface would promote the functional integration of cartilage grafts[2]. We have designed a hydrogel-ceramic composite scaffold for interface formation, as the ceramic phase mimics the mineral of the calcified cartilage region and hydrogels such as agarose have been successfully utilized for cartilage tissue engineering[3,4]. The **objective of this study** is to examine and compare the effect of two types of calcium phosphate ceramics, β-tricalcium phosphate (TCP) and hydroxapatite (HA), on deep zone chondrocytes (DZC) response in the hydrogel-ceramic scaffold. It is well established that TCP is more bioactive and biodegradable than HA[5]. It is anticipated that ceramic bioactivity will modulate chondrocyte growth, biosynthesis and deposition of a calcified cartilage-like matrix.

Methods: DZC were isolated from the bottom 30% of neonatal calf cartilage tissue[6] and maintained in ITS media with 50 µg/mL ascorbic acid. Cells combined with 1.5% w/v HA (Sigma) or β-TCP (Sigma) were mixed with 4% agarose (10 million cells/ml). The ceramic phase within the hydrogel was characterized by EDAX, SEM and FTIR (Fig. 1). *Experimental groups* were stimulated with 25 nM triiodothyronine (T3), a known promoter of hypertrophy[7] for the first 6 days of culture. *Control groups* included DZC in T3-unstimulated, ceramic-free scaffolds and corresponding acellular scaffolds. Samples were analyzed on Days 1, 7, and 14 for DNA (n=5), alkaline phosphatase activity (ALP,n=5), and production of glycosaminoglycans (GAG, n=5) and collagen (n=5) with corresponding histology (n=2). Additionally, gene expression (n=3) for collagen X, PthrP, Ihh, and MMP13 was determined by RT-PCR, with GAPDH 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)[8]. Statistical analysis: ANOVA and Tukey–Kramer tests were performed ($p<0.05$).

Results: A significant increase in cell number is observed by day 7 for both ceramic groups, however by day 14 the TCP group measured a significantly higher cell number than both the control and HA groups (data not shown). Extensive matrix deposition is observed over time for all groups, with significantly higher collagen deposition for both ceramic groups at Day 14 and significantly higher GAG deposition by Day 14 in the TCP group when compared to all other groups (Fig. 2). As expected, T3 enhances chondrocyte ALP activity and the expression of hypertrophic markers, although ALP is suppressed with the addition of TCP in the T3 stimulated group at day 14 (Fig. 3). Also evident is an early ALP enhancement in the presence of TCP that is independent of T3 stimulation. Cell-seeded scaffolds exhibit higher shear mechanical properties than the acellular controls due to matrix elaboration. All groups exhibit a higher dynamic shear modulus and significant increase in elastic modulus in all groups by day 14 (Fig. 4).

Discussion: These results demonstrate that changes in DZC biosynthesis and mineralization are dependent on ceramic activity, with the presence of TCP stimulating the formation of a calcified matrix rich in collagen and proteoglycans that resembles the osteochondral interface. Our results suggest that while both inert (HA) and active (TCP) ceramics stimulate DZC biosynthesis, there is enhanced biosynthesis with TCP. This increased biosynthesis is likely due to the bioactivity of the TCO which modulates DZC response. Future studies will focus on elucidating the cause for increased biosynthesis in the presence of bioactive TCP.

References: 1) Bullough et al, J Bone Joint Surg Br, 1983 2) Hunziker et al, Clin Orthop Relat Res, 2001. 3) Mauck et al., Biomech Model Mechanobiol.,2006. 4)Cook et al, Am J Vet Res 1997. 5) LeGeros, Chem. Rev. 2008 6) Jiang et al, Osteoarthritis Cartilage. 2008. 7) Rosenthal et al., J Rheumatol. 1999. 8) Zhu et al, J. Orthop. Res. 1993. **ACKNOWLEDGMENT:** NIH-NIAMS 5R01AR055280



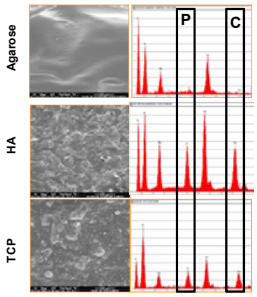
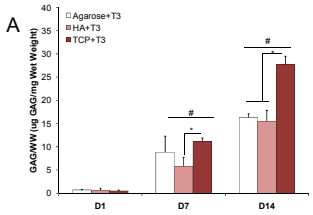
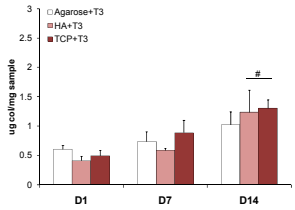


Figure 1. Scaffold Characterization. FTIR spectra of both HA and TCP and SEM images of as-fabricated scaffolds with corresponding EDAX (right)analysis.





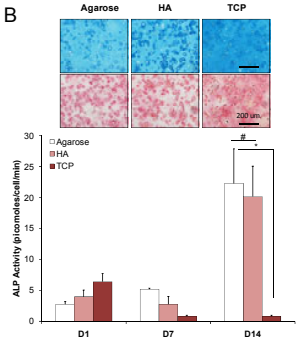


Figure 2. Matrix Deposition. A) Quantitative GAG (left) and collagen (right) deposition B) Alician Blue on top panel and Picrosirius Red on bottom panel

Figure 3. Mineralization. ALP Activity of T3-stimulated groups

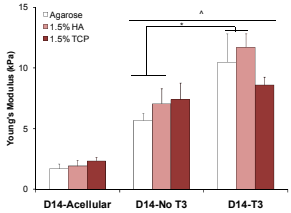


Figure 4. Mechanical Properties. Elastic Modulus on Day 14 for cell-seeded samples