Effects of Ceramic Structural Properties on Chondrocyte Response

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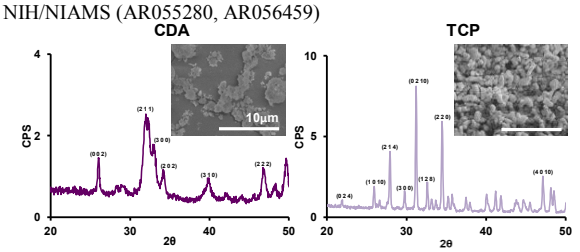
Statement of Purpose: Osteoarthritis is characterized by cartilage degeneration and is the leading cause of physical disability among Americans. Repair of cartilage defects currently does not result in the regeneration of a calcified cartilage region. This region provides a barrier to prevent osseous upgrowth, thus supporting the integrity of the cartilage compartment during healing[1]. Regeneration of this interface is critical to promote the stable integration of cartilage grafts. To this end, we have designed a hydrogel-ceramic composite scaffold for calcified cartilage formation, whereby the ceramic phase mimics the mineral of the calcified cartilage region and hydrogel phase mimics the high water content of cartilage tissue. The **objective of this study** is to optimize the ceramic phase by determining the effect of ceramic structure on chondrocyte growth and biosynthesis. Specifically, cell response to calcium-deficient apatite (CDA) and beta-tricalcium phosphate (β-TCP) are compared. CDA was sintered to obtain β-TCP. It is hypothesized that the difference in crystal structure between CDA and β-TCP will modulate chondrocyte growth and the deposition of a calcified cartilage-like matrix.

Methods: *Ceramic:* CDA powder (Sigma) was sintered at 900°C for 3 hours to produce β-TCP. CDA and β-TCP were characterized via SEM, XRD, FTIR, and ICP. *Cell culture and Scaffold Fabrication:* Deep zone chondrocytes (DZC) were isolated from the bottom 30% of immature femoral calf cartilage[2] and maintained in ITS media with 50 µg/mL ascorbic acid. Cells in agarose were combined with CDA or β-TCP for a final concentration of 10 million cells/ml, and 1.5% w/v ceramic in 2% agarose. DZCs in agarose served as the control group. *End-point Analyses:* Samples were analyzed at 3hr, 8hr, and 1, 7, and 14 days for DNA (n=5), alkaline phosphatase activity (ALP, n=5), media ion concentrations (n=6), glycosaminoglycans (GAG, n=5) and collagen content (n=5) and corresponding histology (n=2). *Statistical Analysis:* ANOVA and Tukey–Kramer post hoc tests were performed to determine statistical differences (*p<0.05 between groups, #p<0.05 over time).

Results: Characterization analysis revealed that CDA exhibited a small apatite crystallite size, poorly defined particle shape, and a Ca/P molar ratio of 1.40±0.02. FTIR spectra revealed the presence of hydroxyl and carbonate groups. Sintering of CDA resulted in the formation of β-TCP as verified by XRD, with a Ca/P molar ratio of 1.41±0.02 and a rhombic particle shape (Fig 1). In terms of cell response, cell number increased over time in all groups with the highest cell number observed in the CDA group first at day 7 and also at day 14 (p<0.05). Significantly higher GAG deposition was found in the CDA group with respect to the control at day 7 and the highest GAG and collagen deposition was detected in the CDA group at day 14. Media calcium concentration was the lowest in (*p<0.05) the CDA group at day 1, whereas media phosphate concentration was the highest in the β-TCP group (Fig 2). ALP activity was elevated for both ceramic groups at early time points with significantly higher ALP activity found in the CDA group (Fig 3).

Discussion: The results of this study suggest that DZC biosynthesis and mineralization are dependent on ceramic structure, with the presence of CDA stimulating the formation of a calcified matrix rich in collagen and proteoglycans that is similar to the matrix of the calcified cartilage[3]. The presence of a ceramic phase induced an early peak in ALP activity, with the highest enzyme activity associated with the CDA group. These differences may be related to bioavailability of calcium ions[4]. While CDA stimulates DZC proliferation and biosynthesis, no such effect was evident with β-TCP. These variations in cell response may be attributed to the differences in the chemistry and crystal structure between CDA and β-TCP which affect dissolution rates and ion release[5,6]. Future studies will focus on elucidating the mechanism by which crystal structure affects cell response.

References:1) Hunziker et al, Clin Orthop Relat Res, 2001. 2) Jiang et al, Osteoarthritis Cartilage. 2008. 3)Khanarian et al, ORS 2012 4) Khanarian et al, Tissue Eng. 2012 5) Wang et al, Journal of Biological Chem, 2001 6)LeGeros Clinical Materials 1993 **Acknowledgments:** NIH/NIAMS (AR055280, AR056459)



	Ca (wt%)	P (wt %)	Ca/P Molar Ratio
CDA	31.58 ± 0.36	17.35 ± 0.14	1.41 ± 0.02
TCP	33.54 ± 0.40	18.56 ± 0.18	1.40 ± 0.02

Figure 1. CDA characterization, before and after sintering

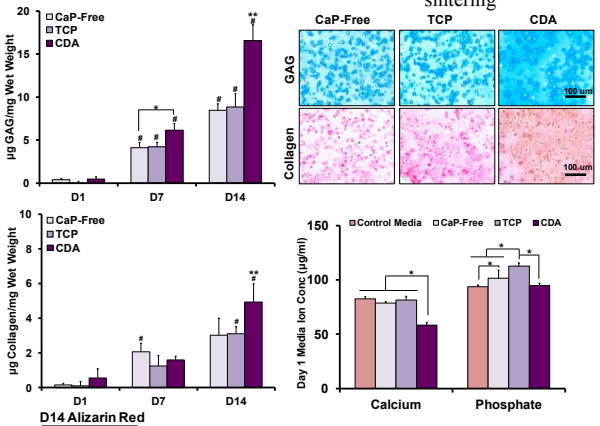


Figure 2 (above). Matrix deposition and media content.

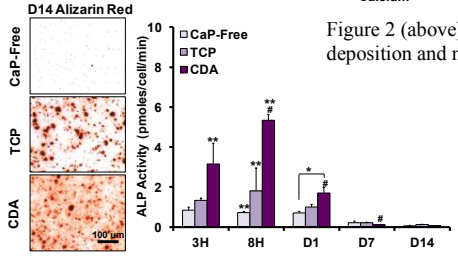


Figure 3 (left). Alizarin Red (10x) and ALP Activity.