Dose-Dependent Effects of CDA on Chondrocyte Response on Polymer-Ceramic Composite Scaffolds

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Statement of Purpose: Tissue engineered grafts represent a promising approach to cartilage repair; however, the regeneration of a stable calcified cartilage layer between newly generated cartilage and bone remains a clinical challenge [1]. Articular cartilage integrates with bone via a calcified cartilage interface that functions as a barrier to osseous upgrowth during cartilage healing [2]. Regeneration of this interface is critical for integrative cartilage repair. Our prior study revealed that calcium deficient apatite (CDA) nanoparticles incorporated in agarose hydrogel facilitated the formation of a calcified cartilage-like matrix [3]. Building on these findings, we sought to optimize a biodegradable system for calcified cartilage repair using CDA. To this end, electrospinning is a versatile fabrication technique that can be used to generate porous scaffolds with high surface area [4]. The **objective of this study** is to investigate chondrocyte response to polymer microfibers as a function of CDA dose (10-20%, w/w). Deep zone chondrocytes (DZCs) are used because they are located closest to the osteochondral interface. **It is hypothesized** that changes in the CDA dose will modulate DZC matrix deposition and mineralization.

Methods: *Scaffold Fabrication:* Unaligned microfiber scaffolds composed of a 5:1 blend of PLGA poly(lactide-*co*-glycolide) (PLGA 85:15, Lakeshore) and poly(ε-caprolactone) (PCL, Sigma) were fabricated with 0, 10, 15, and 20% (w/w) CDA nanoparticles (Sigma) via electrospinning [4]. The molar Ca/P ratio of the CDA was characterized using inductively coupled plasma (ICP) analysis (n=6). Scaffolds were imaged using scanning electron microscopy (SEM) and fiber diameter was measured (ImageJ). *Cells and Cell Culture:* DZCs were isolated from the bottom 30% of femoral calf cartilage and seeded onto scaffolds after 72 hours of monolayer culture. Cell-laden scaffolds were maintained in ITS media with 50 µg/mL ascorbic acid. *Cell Growth, Biosynthesis, and Mineralization:* Total DNA, glycosaminoglycan (GAG) and collagen production were measured using the Picogreen dsDNA assay (Molecular Probes), the dimethylmethylene blue dye-binding assay and a modified hydroxyproline assay, respectively (n=5). Alkaline phosphatase (ALP) activity (n=5) was detected using a colorimetric assay. Von Kossa, alcian blue and picrosirius red were used to stain for mineral, GAG and collagen, respectively (n=2). *Statistical Analysis:* ANOVA and the Tukey-HSD test was used ($p<0.05$).

Results: *Scaffold Characterization*: A Ca/P molar ratio of 1.41 ± 0.02 for the CDA ceramic was determined using ICP analysis. SEM revealed a comparable mean fiber diameter for all scaffold groups (1.1-1.4 µm). *Cell Response:* Chondrocytes remained viable and proliferated over time in all groups with more cells on day 7 than day 1 ($p<0.05$). By day 42, the 20% CDA group had higher cell number than the ceramic-free control ($p<0.05$). The highest ALP activity was detected for the 10% group on day 1, with lower ALP activity for all groups after day 1 ($p<0.05$, Fig 1). *Matrix Deposition:* While higher GAG was detected in the 15% and 20% groups on day 42, the highest GAG content was measured for the 20% group ($p<0.05$). The highest collagen production at day 14 was found in the 10% CDA group; however, by day 42, the 20% CDA group measured the highest collagen content. These observations were confirmed via histology (Fig 2).

Conclusions: Our results demonstrate that chondrocyte response is modulated by CDA dose within polymer microfibers. Specifically, 20% CDA in a PLGA:PCL microfiber system resulted in enhanced GAG and collagen production compared to lower doses, similar to findings in agarose, in which matrix deposition was a function of ceramic dose. It is envisioned that this scaffold can be used to augment current cartilage grafts by acting as a temporary barrier during cartilage repair and provide a template for stable osteochondral interface regeneration.

References: [1]Lu et al., 2010. [2]Hunziker et al., 2001.[3]Boushell et al., 2012. [4]Moffat et al, 2009.

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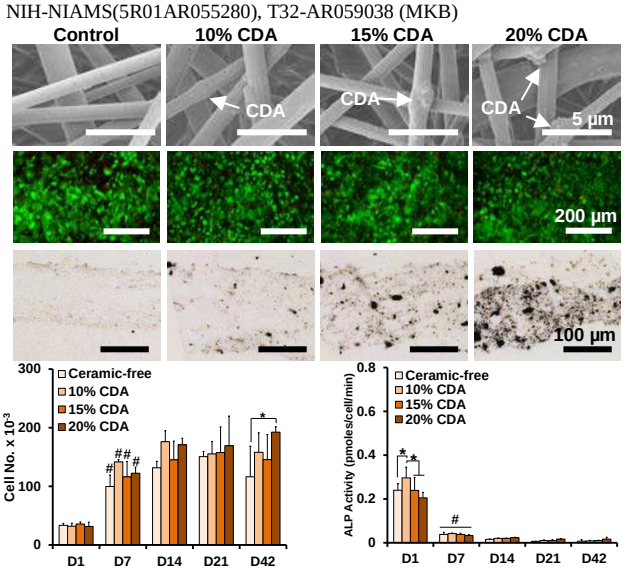

Figure 1. Top: SEM, Middle: D42 Live/Dead, Bottom: D42 Von kossa. Cell number and ALP activity (*$p<0.05$ between groups, #$p<0.05$ over time)

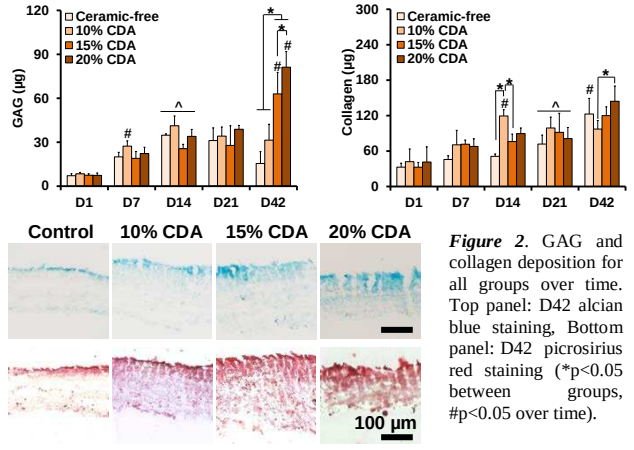

Figure 2. GAG and collagen deposition for all groups over time. Top panel: D42 alcian blue staining, Bottom panel: D42 picrosirius red staining (*$p<0.05$ between groups, #$p<0.05$ over time).