Cationic Contrast-Enhanced Computed Tomography Biomarkers Distinguish Reparative and Degenerative Articular Cartilage in an Equine Model

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Introduction: Osteoarthritis (OA) is a progressive joint disease that degrades articular cartilage. Most radiographic methods are insensitive to detect early changes in cartilage associated with OA and are unable to characterize healing tissue. Cationic contrast-enhanced computed tomography (CCECT) improves cartilage evaluation by using an intra-articular cationic (CA4+) contrast medium to partition into the cartilage in direct proportion to glycosaminoglycan (GAG) concentration so that the CT attenuation reflects the biochemical composition that accounts for the mechanical performance of normal and degenerative articular cartilage in bovine and equine explants.[1] However, the ability of CCECT to differentiate reparative, degenerative and normal articular cartilage is unknown. We hypothesize that CCECT attenuation can represent cartilage GAG concentration and equilibrium compressive modulus that distinguishes reparative from degenerative and normal articular cartilage.

Methods: All procedures were reviewed and approved by the Animal Care and Use Committee at Colorado State University. Seven horses were anesthetized and one femoropatellar joint in each horse was randomly assigned to receive cartilage defects (2 – circular, 15 mm diameter) on the medial trochlear ridge of the femur. One defect had calcified cartilage (CC) retained (Reparative cartilage 1, R1) and the other had CC removed (Reparative cartilage 2, R2). The contralateral (control) joint was sham-operated to confirm normal articular cartilage. Four horses were sacrificed at 2 months and three horses were sacrificed at 4 months after defect creation. Fourteen osteochondral biopsies (circular, 7 mm diameter) were collected from articular surface locations in each defect joint and were denoted as R1 (n=2), R2 (n=2), adjacent (n=4), or remote (n=6). Adjacent (to defect) osteochondral biopsies abutted the R1 or R2 defects, and represented degenerative tissue (defect joints only). Remote biopsies were collected >15 mm from the defects. These same designations were used in the control joints. Each biopsy was graded using the International Cartilage Repair Society (ICRS) scale.[2] Mechanical testing (unconfined 4-step stress-relaxation compressive regimen with incremental 5% strain steps) was performed to determine equilibrium compressive modulus (EM). Insufficient geometry limited mechanical testing to adjacent and remote samples (n=142). After saline equilibration, osteochondral biopsies were submerged in CA4+ (24 mg I/mL, 400 mOsm/kg) at 25°C for 24 hours and imaged with microCT (n=196). Articular cartilage was segmented (semi-automatically) from the subchondral bone and CCECT x-ray attenuation recorded. After saline washout to remove CA4+, the cartilage was removed and analyzed for GAG content using a 1,9 dimethylmethylene blue assay (n=196). Comparisons of CCECT attenuation to EM and GAG were made using Spearman's rank correlation and results were analyzed at each of the four locations and comparisons made between joints and across locations using a mixed model ANOVA. Pairwise comparisons were made after Tukey adjustment. Significance was set at P<0.05 (SAS, Cary NC).

Results: In the defect joints, R1 samples had minimal (ICRS 3-4) and R2 samples had moderate (ICRS 2-3) amounts of tissue filling the defects. Cartilage was macroscopically normal in the adjacent (ICRS 0-1) and remote (ICRS 0) biopsies from the defect joints and at all locations in the control joints (ICRS 0). There was no macroscopic difference in cartilage between the 2 and 4 month time points. CCECT distinguished among the biopsy tissue groups: R2, adjacent and remote **(Figure 1)**. CCECT attenuation strongly correlated with GAG concentration (ρ=0.75, P<0.0001) and EM (ρ=0.77, P<0.0001) **(Figure 2)**. The preload used for mechanical testing in adjacent and remote samples overwhelmed the repair tissue (R1, R2) and thus the EM data reflected the subchondral bone and was excluded from the analysis. Cartilage biopsy location and defect versus control joint influenced CCECT attenuation (P<0.0001), but not in remote biopsy locations.**(Figure 3)**. CCECT attenuation in the R1 and R2 groups were each significantly different from adjacent and remote cartilage (all P<0.0001) but underpowered to distinguish between R1 and R2 (P=0.08).

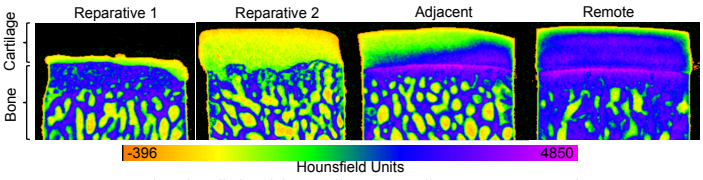

Figure 1: CCECT imaging distinguishes cartilage across disease states. Reparative 1&2 - fibrocartilage; Adjacent – degenerative cartilage; Remote – healthy cartilage. Note the increasing CCECT signal from reparative 2 to adjacent and remote locations. Reparative 1 CCECT attenuation was slightly higher (green-yellow) than reparative 2 (mostly yellow).

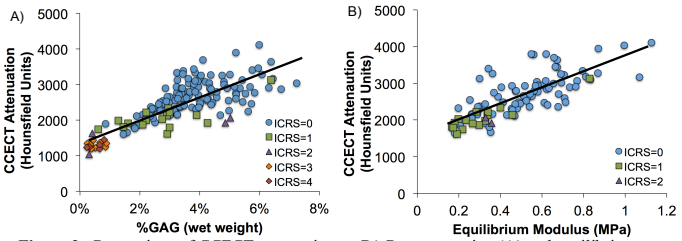

Figure 2: Comparison of CCECT attenuation to GAG concentration (A) and equilibrium compressive modulus (B) in varying cartilage disease states grouped by ICRS score.

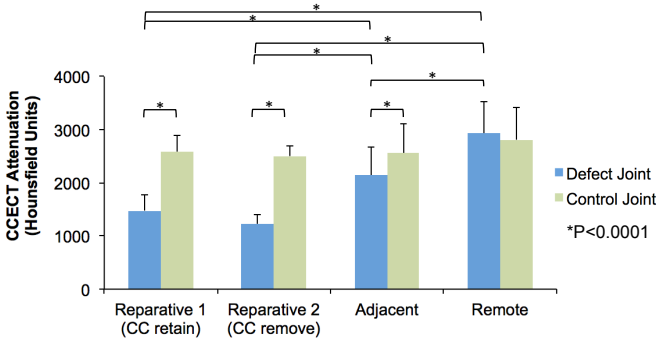

Figure 3: Mean ± standard deviation CCECT attenuation at cartilage locations in control and defect joints. Reparative 1 (calcified cartilage [CC] retained) and reparative 2 (CC removed) groups reflect fibrocartilage and the adjacent group reflects degenerative cartilage in defect joints. In control joints, these locations represent normal cartilage at the same locus to account for the variability across the articular surface.

Discussion: These results show that CCECT attenuation reflects different cartilage disease and reparative states. CCECT attenuation indicates the biochemical and mechanical properties of degenerative, reparative (fibrocartilage) and normal cartilage. Biochemical responses in cartilage adjacent to full thickness defects have been documented[3] and this study shows CCECT depicts these same alterations through non-destructive assessment. Owing to the low number of horses, the study was underpowered to distinguish CCECT attenuation between R1 and R2. Further experiments are required to determine the ability of CCECT to distinguish among disease and reparative states *in vivo* and in non-equine disease models.

Significance/Clinical Relevance: CCECT is capable of discerning reparative from degenerative and normal articular cartilage and has potential as a method to monitor healing cartilage tissue in longitudinal evaluations.

References: 1) Bansal, P.N. *et al. Osteoarthritis Cartilage* 18, 184-191 (2010); 2) Brittberg, M. *et al. J Bone Joint Surg Am* 85(Suppl 2), 58-69 (2003); 3) Strauss, E. J. *et al. Am. J. Sports Med.* 33, 1647–1653 (2005)

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