Contrast Enhanced Computed Tomography of Equine Joint Cartilage Demonstrates Consistent Imaging Relationships Across Joint Surfaces

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Statement of Purpose: In the context of evaluating cartilage diseases, such as osteoarthritis (OA), there is a need to develop minimally invasive methods that can accurately measure the biochemical properties of articular cartilage. Currently, contrast enhanced imaging of cartilage by MRI and CT (CECT) using anionic contrast agents have been demonstrated to measure the glycosaminoglycan (GAG) content of articular cartilage predicated on the diffusion and equilibrium partitioning of the charged contrast agent into the cartilage[1]. The fixed negative charge density produced by the GAGs comprising the extracellular matrix (ECM) maintains cartilage hydration and affords its compressive stiffness, and GAG loss is indicative of early OA. Recently, a cationic iodinated contrast agent bearing 4 positive charges (CA4+) has been developed which exploits electrostatic attraction to the GAGs, providing a proportionate relationship between cartilage GAG content and CECT attenuation with improved sensitivity to changes in GAG content and at lower concentrations compared to commercially available anionic contrast agents[2,3]. In this study, we used CECT with CA4+ to examine the consistency of the relationship between equilibrium CECT attenuation and GAG content across different joint surfaces in an equine osteochondral plug model.

Methods: Both stifle joints of a 2 year old horse without joint disease were utilized. Seventy-three cartilage plugs (7 mm diameter) were harvested from multiple surfaces of each stifle joint (femoral condyles, femoral groove, and patella). The plugs were immersed in CA4+ (8 mgI/mL) for 24 hours and scanned (microCT40, Scanco Medical) at an isotropic voxel resolution of 36 μm^3, 70 kVP tube voltage, 113 µAmp current, and 300 ms integration time. The CT data sets were imported into Analyze™ (BIR, Mayo Clinic) and the cartilage segmented from the subchondral bone using a semi-automatic contour based algorithm. The cartilage was removed from the subchondral bone and GAG content was determined using the 1,9-dimethylmethylene blue colorimetric assay. Univariate linear regression was used to express CECT attenuation (Hounsfield Units, HU) as a function of plug GAG content (mg/mg wet weight) for each individual joint surface (SPSS Statistics v17.0, Chicago, IL). ANCOVA was used to compare the model parameters for each regression.

Results: For both stifle joints, differences in the slopes among the linear regressions for plugs from the patella, femoral condyles, and femoral groove were not statistically different (p=0.30 for the right stifle, p=0.10 for the left) (**Figure 1 top and bottom**).

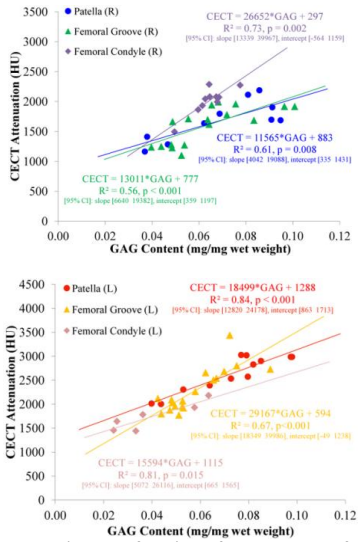

Figure 1. CECT attenuation as a function of GAG content for surfaces in the right (**Top**) and left (**Bottom**) stifle. ANCOVA failed to detect significant differences between the regressions (p=0.30 for right, p=0.10 for left).

Conclusions: Previous studies have shown the capacity of CECT using CA4+ and commercially available anionic contrast agents to measure the GAG content in osteochondral plug models[1-3]. However, it has been suggested that other cartilage ECM features in addition to GAG influence the partitioning of the contrast agent even at equilibrium [4,5]. Since ECM differences are likely to occur in different joints, between opposing joint surfaces, and among individuals, we compared the CECT vs. GAG relationships among different joint surfaces and found that these relationships were similar within a joint, suggesting that CECT could be used as a reliable tool to evaluate the GAG content of multiple joint surfaces. We are currently evaluating multiple joint samples excised from additional horses to investigate the consistency of these relationships and whether CECT can be used to evaluate any joint surface with relatively equivalent accuracy. For CECT to be useful clinically, consistent relationships between CECT attenuation and cartilage GAG should be demonstrated. Given the variability in multiple ECM components and their potential impact on contrast uptake, we have demonstrated that for the equine stifle joint, these relationships are consistent; thus, CECT may be feasible for comparing cartilage quality across multiple joint surfaces.

References: (1) Palmer, A. et al., *PNAS* 2006. (2) Bansal, P. et al., *Osteoarthr Cartilage* 2011. (3) Stewart, R. et al., *Radiology* 2013. (4) Benders, K. et al., *Osteoarthr Cartilage* 2010. (5) Wiener, E. et al., *Muskuloskelettales System* 2010.