Articular Cartilage Characterization Using Tantalum and Iodine-based Dual-Contrast Photon-Counting Computed Tomography

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INTRODUCTION: Photon-counting detector computed tomography (PCD-CT) is a novel spectral CT imaging technique. PCDs detect individual photons and classify them into fixed energy bins, thus enabling spectral imaging, and e.g., separation of multiple contrast agents from a single scan. In this study, using non-ionic iodinated iodixanol and cationic tantalum oxide nanoparticles (TaO-NPs), we employ a dual-contrast PCD-CT technique to assess structural and biomechanical characteristics of equine articular cartilage.

METHODS: *Samples:* Intercondylar notch (n = 15) and medial femoral condyle (n = 15) articular cartilage samples (cylindrical plugs, d = 8.5 mm, n = 30) were extracted from equine stifle joints (acquired from an abattoir, N = 15). The plugs were split into quarters: three quarters of each plug were used for reference methods and one quarter of each plug was immersed for 96 h (T = 37 °C) in a dual contrast agent mixture of cationic TaO-NPs (Ta_2O_5, 20 mg·Ta_2O_5/mL) and non-ionic iodixanol (Visipaque™, 40 mg·I/mL) (Fig. 1A). Non-articulating surfaces were sealed with cyanoacrylate before the immersion. *PCD-CT:* The setup consisted of PCD (XC-Flite FX15, XCounter AB), motorized rotator (NR360S, Thorlabs Inc.), and mini-focus X-ray source (IXS1203MF, VJX) with 3 mm aluminum and 0.5 mm copper filters. The used tube voltage was 120 kVp and the used low and total energy bins were 10-80 keV and 10-120 keV, respectively. The samples were imaged before the immersion (0-hour) and at a 96-hour timepoint (Fig. 1B). Calibration of the concentration estimation was based on separately scanned solutions with ten different contrast agent concentrations. Voxel size was 68 × 68 × 68 µm^3 in the reconstructed volume. *Biomechanics:* Before splitting the plugs, a four-step stress-relaxation protocol (step size: 4% of remaining cartilage thickness, relaxation: 10 min) and sinusoidal dynamic test (strain amplitude: 4% of remaining cartilage thickness, frequency: 1 Hz, cycles: 4) were conducted with a custom-made biomechanical tester fitted with a 0.25 kg load-cell (Model 31, Honeywell International Inc.) and a flat-ended cylindrical indenter (d = 0.55 mm). Hayes corrected equilibrium modulus, strain-dependent instantaneous modulus, and dynamic modulus were calculated (E_{Eq}, E_{Inst}, and E_{Dyn}, respectively). *Proteoglycan content:* Spatial proteoglycan (PG) content was determined from Safranin-O-stained sections using digital densitometry. *Analysis of CT images:* Calibration coefficients were calculated from fits of the measured attenuation values of the calibration solutions. Then a custom-made MATLAB (R2020b, MathWorks) code was used to estimate the contrast agent concentrations in the cartilage of the low and total energy bin reconstructions (Fig. 1B) [1]. Subsequently, depthwise contrast agent partition (i.e., ratio against the original bath concentration) profiles and bulk (full-thickness) cartilage partition values were calculated (Fig. 1C). *Statistics:* Spearman's correlation coefficients (r) between bulk contrast agent partitions and reference data were calculated with MATLAB.

RESULTS: Concentration estimation was validated with nine different concentration mixtures of contrast agents: mean errors were 18.7% and 8.3% for TaO-NP and iodixanol, respectively. Depthwise partition profiles were determined for all samples and mean (±standard deviation) partition profiles were calculated (Fig. 1C). The bulk TaO-NP partition correlated positively with E_{Eq} and E_{Dyn} (Table 1). The bulk iodixanol partition had a strong negative correlation with all the biomechanical moduli. No significant correlation was detected between PGs and the contrast agents alone, but when TaO-NP partition was normalized (divided) with iodixanol partition, a significant positive correlation was detected. Normalization also improved the correlation with biomechanical moduli, and a moderate correlation was observed between normalized TaO-NPs and E_{Inst}.

DISCUSSION: Herein we describe the first use of dual-contrast PCD-CT imaging using TaO-NPs and iodixanol to correlate contrast agent partition with cartilage biomechanics and composition. The dual-contrast method reveals that the partition of cationic TaO-NPs correlates positively with E_{Eq}. This was expected, as cationic contrast agents are known to bind to anionic PGs [1-3], which mainly determine cartilage equilibrium stiffness. Even though the collagen network inhibits TaO-NPs diffusion [2], a positive correlation was found between TaO-NP partition and E_{Dyn}, which can be explained by healthy cartilage and cross-correlation. Iodixanol partition showed a significant negative correlation with all the biomechanical moduli. As it is a non-ionic contrast agent and its diffusion is mainly driven by osmotic pressure, the water content in the cartilage dominates the uptake. The normalization of the TaO-NP partition with the iodixanol partition greatly improved the correlations. Authors believe that TaO-NPs are large enough to affect the iodixanol diffusion in the cartilage, i.e., the rate of iodixanol diffusion is decreased in the presence of TaO-NPs. Thus, the normalization with iodixanol could be used to improve the sensitivity of TaO-NPs, i.e., iodixanol's faster diffusion might enhance the performance of normalized TaO-NPs in earlier timepoints.

SIGNIFICANCE/CLINICAL RELEVANCE: We demonstrate that the dual-contrast PCD-CT method, utilizing cationic TaO-NPs and non-ionic iodixanol, allows biomechanical and structural evaluation of articular cartilage. Especially, the normalization of the TaO-NPs with iodixanol improves the accuracy of the evaluation.

REFERENCES: [1] Paakkari et al. (2021) Sci. Rep. 11:5556, [2] Jäntti et al. (2022) Osteoarthr. Cartil. 30:S277, [3] Lawson et al. (2021) ACS Nano. 15:19175-19184

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Table 1: Spearman's correlation coefficients between bulk cartilage contrast agent partitions, and biomechanical moduli, and proteoglycan (PG) content.

	Iodixanol	TaO-NP	TaO-NP normalized
Equilibrium Modulus (E_{Eq})	**-0.488****	**0.531****	**0.668*****
Instantaneous Modulus (E_{Inst})	**-0.504****	0.360	**0.511****
Dynamic Modulus, 1 Hz (E_{Dyn})	**-0.622*****	**0.555****	**0.702*****
PG-content	-0.426	0.395	**0.552***

Statistical significance: * p<0.05; ** p<0.01; *** p<0.001

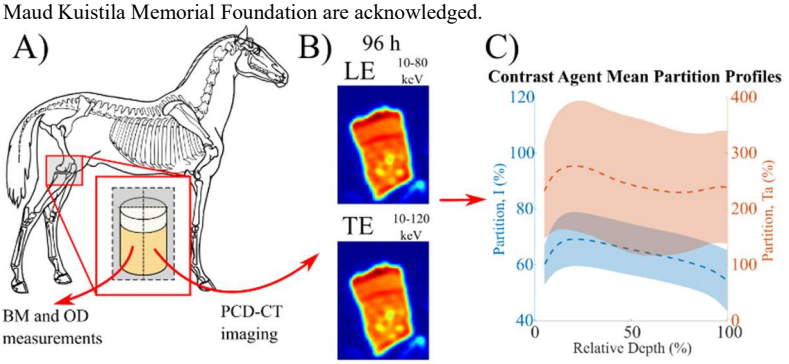

Figure 1: A) Cylindrical plugs (n = 30) were extracted from equine stifle joint. Each plug was split to quarters after biomechanical (BM) measurements: one quarter was used for PCD-CT imaging and the rest were used for reference methods, e.g., digital densitometry (DD). **B**) Low and total energy bin (LE and TE, respectively) reconstructions were used to determine the contrast agent partitions at 96-hour timepoint. **C**) Depthwise partition profiles for the contrast agents were extracted (I = iodixanol, Ta = tantalum oxide nanoparticles). 0% is the articular cartilage surface and 100% is the cartilage-bone interface.