Synergistic Contrast Agent Approach for Early Osteoarthritis Differentiation Using Tantalum Nanoparticles and Iodine Computed Tomography

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INTRODUCTION: Photon-counting detector computed tomography (PCD-CT) is a novel spectral CT technique that differentiates materials utilizing one X-ray spectrum. We have developed a dual contrast agent method where cationic nanoparticles and non-ionic contrast agent functions together to provide synergistic benefits beyond what is achievable with a single contrast agent. In this study, we first assess the sensitivity of the dual-contrast PCD-CT method to diagnose early osteoarthritis (OA) by detecting significant differences in cartilage contrast agent partitions between healthy and early OA cartilage samples, as classified by Mankin scoring. Second, we investigated the reciprocal influence of these contrast agents on each other's diffusion patterns. This investigation holds importance in elucidating the dynamics that underlie nanoparticle-molecule interactions, and aids in refining and advancing this methodology.

METHODS: _Dual-Contrast Method for Early OA Detection: Samples:_ Distal intertrochlear groove ($n = 15$) and medial femoral condyle ($n = 15$) articular cartilage samples (cylindrical plugs, $d = 8.5$ mm) were extracted from equine stifle joints ($N = 15$). **Contrast Agents:** A dual contrast agent bath ($T = 37$ °C) containing of cationic tantalum oxide nanoparticles (Ta_2O_5-NPs, 20 mg·Ta_2O_5/mL) and non-ionic iodixanol (Visipaque™, 40 mg·I/mL) was used. Non-articulating surfaces had been sealed with cyanoacrylate. **PCD-CT:** An experimental tabletop PCD-CT system was used to image samples at different timepoints (0-96 hours). Tube voltage was set to 120 kVp with 3 mm aluminum and 0.5 mm copper filters. To allow spectral imaging, low and total energy bins of the detector were set to 10-80 and 10-120 keV, respectively. Voxel size was $68 \times 68 \times 68$ µm^3 in the reconstructed spectral bin volumes. **Histological grading:** Safranin-O-stained histological sections were prepared, blind-coded and graded by four independent assessors using the Mankin score. Two groups were formed: healthy (Mankin grades 0-1, $n = 10$) and early OA (Mankin grades 2-4, $n = 20$). _Sequential Dual-Contrast Diffusion Experiment: Samples:_ Lateral trochlear ridge ($n = 2$) and medial femoral condyle ($n = 2$) articular cartilage samples (cylindrical plugs, $d = 8.5$ mm) were extracted from equine stifle joints ($N = 2$) and cut into halves to form two groups, both with four samples. **Contrast Agents:** Samples were first immersed in a single contrast agent bath (one group in a Ta_2O_5-NP bath and other group in an iodixanol bath) and then in a dual contrast agent bath (both groups in a Ta_2O_5-NP and iodixanol bath) to study the contrast agent interactions in cartilage. Concentrations of the baths were kept the same (20 mg·Ta_2O_5/mL and 40 mg·I/mL). **Micro-CT:** Samples were imaged with a conventional micro-CT scanner at different timepoints (0-144 hours) using a dual-energy protocol with two scans (80/130 kVp) and voxel size of $25 \times 25 \times 25$ µm^3. _Image analysis:_ A custom-made MATLAB (R2020b, MathWorks) code was used for analysis. Contrast agent concentration estimation was derived from individual calibration solutions (10 concentrations) [1]. Full cartilage thickness partitions, _i.e.,_ ratio against the original bath concentration, were calculated for both contrast agents, and Ta_2O_5-NP partition was normalized (_i.e._, Ta_2O_5-NP partition was divided by iodixanol partition). _Statistics:_ A Kruskal-Wallis test was used to assess contrast agent partition differences between healthy and early OA groups.

RESULTS: _Dual-Contrast Method for Early OA Detection:_ The difference in normalized Ta_2O_5-NP partition between healthy and early OA sample groups was significant starting from the 12-hour timepoint ($p = 0.0138$) (Fig. 1A). _Sequential Dual-Contrast Diffusion Experiment:_ Iodixanol exerted an inhibitory effect on Ta_2O_5-NP diffusion (Fig. 1B). In the absence of iodixanol, Ta_2O_5-NP exhibited accelerated and more extensive diffusion. However, Ta_2O_5-NPs' presence did not appear to impact the diffusion of iodixanol (Fig. 1C).

DISCUSSION: This study marks the first report of significant interactions between molecule- and nanoparticle-based contrast agents during their diffusion into articular cartilage, with a notable observation: iodixanol slows Ta_2O_5-NP diffusion (Fig. 1B), while reciprocal impact on iodixanol diffusion remains insignificant (Fig. 1C). This result is attributed to the difference in size between iodixanol molecules and Ta_2O_5-NPs. Analogically, iodixanol is like sand and Ta_2O_5-NPs are like pebbles, and thus, when container is filled with pebbles, there is still space for sand, but vice-versa pebbles will accumulate on top of the sand. However, in our context, the iodixanol does not fully block Ta_2O_5-NP; it decelerates diffusion. This dynamic synergy bears promise for detecting early OA changes in cartilage. By manipulating the interplay between the contrast agents and combining it with PCD-CT, it might be possible to fine-tune the contrast and resolution of the images, leading to clearer and more accurate representations of cartilage structure and properties. In this _in vitro_ study, we observed differences between the normalized Ta_2O_5-NP partitions between healthy and early OA samples, starting at the 12-hour timepoint (Fig. 1A).

SIGNIFICANCE: The study's findings indicate that the introduced dual-contrast PCD-CT method, where non-ionic iodixanol affects the diffusion of cationic Ta_2O_5-NP, differentiates healthy and early OA cartilage. This holds the promise of enhancing articular cartilage imaging and early OA diagnostics.

REFERENCES: [1] Paakkari et al., _Sci. Rep.,_ 11:5556 (2021).
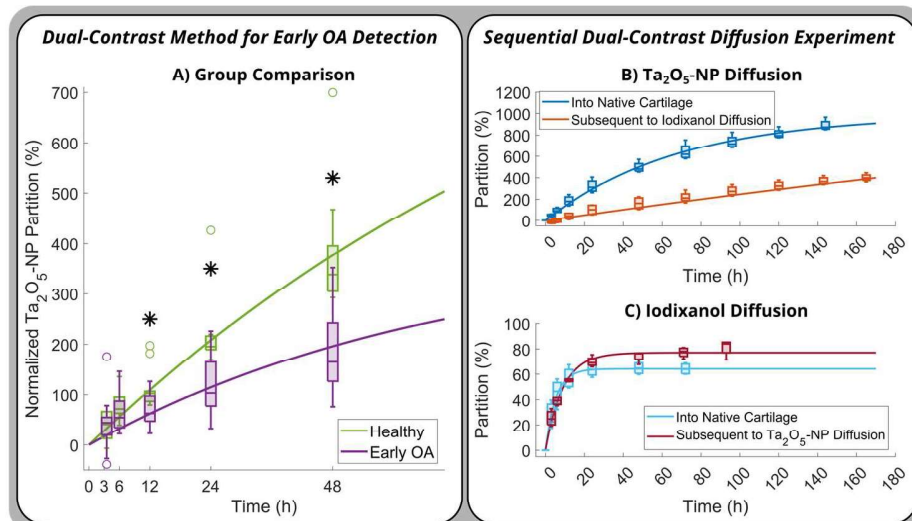
Figure 1: A) Normalized Ta_2O_5-NP diffusion between healthy (Mankin grades 0-1, $n = 10$) and early OA (Mankin grades 2-4, $n = 20$) samples exhibited the differences in diffusion rate. Partition = measured concentration of contrast agent divided by the original concentration in the bath. Normalized Ta_2O_5-NP = Ta_2O_5-NP partition divided by iodixanol partition. Asterisks (*) mark the significant ($p < 0.05$) differences between compared groups. Outliers are marked with circles. **B)** Cationic tantalum oxide nanoparticle (Ta_2O_5-NP) diffusion into native cartilage samples ($n = 4$) and into samples that had been immersed in an iodixanol bath for 72 hours ($n = 4$) revealed the interactions between the two contrast agents. **C)** Non-ionic iodixanol diffusion into native cartilage samples ($n = 4$) and into samples that had been immersed in a Ta_2O_5-NP bath for 144 hours ($n = 4$) showed relatively negligible differences between the diffusions.