Effect of Depth-Dependent Variation in Articular Cartilage Composition on Nanoparticle Diffusion

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INTRODUCTION: Contrast-enhanced computed tomography (CECT) enables imaging of cartilage composition via use of functional contrast agents. Ta_2O_5-NPs are biocompatible, have a good radiopacity, and can be synthesized to be cationic (attracted to proteoglycans) and smaller than the pore size of articular cartilage (AC). Typical osteoarthritic changes include loss of proteoglycans and collagens lead to increased tissue permeability and decreased tissue mechanical integrity. Ta_2O_5-NPs designed to diffuse into AC can distinguish tissue alterations associated with osteoarthritis. This study investigates the depth-wise structure-diffusion relationship.

METHODS: Osteochondral plugs (diameter = 8.5 mm) were harvested from two locations (intercondylar notch n = 15, medial femoral condyle n = 15) of healthy equine stifle joints (acquired from an abattoir) and cut into quarters for CECT diffusion experiments and histological sectioning. The depth-dependent structure of the cartilage was determined using digital densitometry (for proteoglycan content), Fourier-transform infrared spectroscopy (for collagen content), and polarized light microscopy (for collagen parallelism index, i.e., anisotropy, and for collagen orientation angle). The hydrodynamic diameter of the Ta_2O_5-NPs was 3 nm, as characterized by dynamic light scattering. One set of the quartered samples (edges sealed using cyanoacrylate) was immersed in a cationic Ta_2O_5-NP contrast agent bath (30 mg/ml, pH 7.4, 400 mOsm, 37 °C) for 96 h, and imaged with a Nikon XT H 225 µCT device in air before and after 1, 2, 4, 6, 24, 48, 72, and 96 hours (h) of immersion (Figure 1). X-ray attenuation induced by the contrast agent was determined by subtracting baseline attenuation (0 h) from attenuation values at later time points. Depth-wise contrast agent partition profiles were created by dividing the contrast agent attenuation at each timepoint by the bath attenuation. Moreover, a time-dependent partition equation ($Partition(t) = P_{max} \times [1 - \exp(-t/\tau)]$) was used to fit the diffusion data, where P_{max} is estimated partition at the equilibrium, t is the diffusion time, and τ is the time required to reach 63.2% of the P_{max}. This was done separately for each 5% increment of the depth. A Spearman correlation test was used to study the structure-diffusion relationships and the limit of statistical significance was set at $p < 0.05$.

RESULTS: Proteoglycan content correlated positively ($p < 0.05$) with equilibrium partition through the whole tissue (Figure 2). Positive correlation ($p < 0.05$) was found also between collagen orientation angle and equilibrium partition in the superficial (< ~10% depth) and intermediate (~10-30%) zones. Negative correlation ($p < 0.05$) was observed between equilibrium partition and collagen parallelism index (at 1-10% and 35-100%), as well as between the partition and collagen content (at 5-55%).

DISCUSSION: Diffusion of Ta_2O_5-NP contrast agent was seen to be sensitive to changes in superficial zone properties - the zone where osteoarthritis, and the resulting degeneration/alteration of the constituents, initiates. Ta_2O_5-NP contrast agent is also sensitive to proteoglycan loss, the first indication of early osteoarthritis. Increase in collagen content decreases the Ta_2O_5-NP equilibrium partition because collagens limit free fluid volume in cartilage. Increase in collagen fibril orientation angle in superficial cartilage, i.e., disruption of collagen network, leads to higher Ta_2O_5-NP partition. Similarly, the disorganization of the collagen network (parallelism index) is seen as increased Ta_2O_5-NP intake. In the intermediate zone, the collagen fibril orientation being normally random leads to weak parallelism, which is not seen to alter Ta_2O_5-NP equilibrium partition. Since the orientation angle in deep cartilage is constant (perpendicular to the cartilage-bone interface), it cannot account for variations in contrast agent partition. The present results provide valuable information on the relationship between cartilage structure and NP diffusion and demonstrate the feasibility of Ta_2O_5-NPs to detect early indications of osteoarthritis.

SIGNIFICANCE/CLINICAL RELEVANCE: Novel tunable Ta_2O_5-NPs provide a promising platform for early-stage detection of cartilage tissue degeneration.

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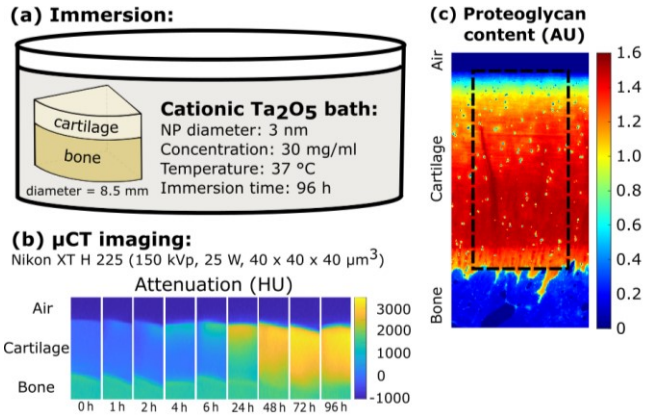

Figure 1: (a) Quarters of healthy osteochondral equine plugs (n = 30) were immersed in Ta_2O_5-NPs bath (edges sealed with cyanoacrylate) for 96 h. (b) The plugs were imaged in air at 0, 1, 2, 4, 6, 24, 48, 72, and 96 h by µCT and attenuation profiles were formed (HU = Hounsfield unit). (c) The depth-dependent proteoglycan content was defined from histological sections (AU = arbitrary unit). The other constituent properties (collagen content, collagen parallelism and orientation angle) were determined similarly.

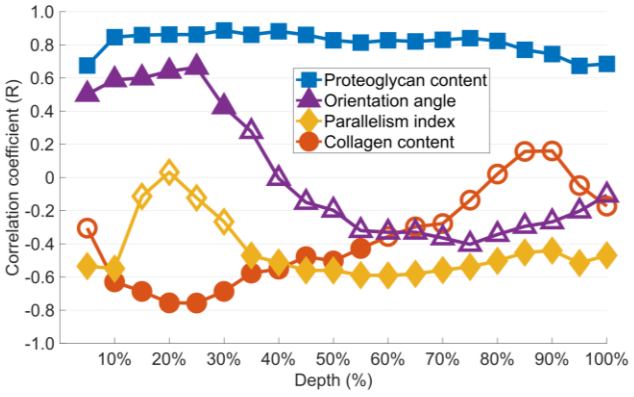

Figure 2: Depth-wise Spearman correlation between Ta_2O_5-NP contrast agent equilibrium partition (P_{max}) and reference methods: proteoglycan content, collagen content, parallelism index, and orientation angle in 5% intervals. 0% depth represents the articular surface and 100% the cartilage-bone interface. Filled markers indicate statistical significance ($p < 0.05$).