Synchrotron µCT Reveals the Potential of the Dual-Contrast Technique for Quantitative Assessment of Human Articular Cartilage Composition

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Introduction: Diffusion of cationic agents (CA) into articular cartilage can sensitively reveal the distribution of negatively charged proteoglycans (PGs) within the tissue1–3. The diffusion is, however, also dependent on the cartilage water content and surface permeability. This complicates the assessment of cartilage composition at clinically feasible diffusion time points, i.e., 1–2 hours after CA administration4,5. As a solution, we recently introduced a dual-contrast CT technique, which utilizes a mixture of cationic and non-ionic CAs, and two X-ray energies (at different sides of a k-edge6). Normalization of the cationic CA distribution (PGs) with that of non-ionic agent distribution (water and permeability) enhances estimation of cartilage PG content2. However, the performance of conventional CT systems is limited by beam hardening, low image resolution (full-body CT), and long acquisition times (conventional µCT). Therefore, to reveal the full potential of the dual-contrast technique for imaging of human cartilage composition, we now employ monochromatic X-ray beam (with two different energies) of a synchrotron µCT for this technique.

Methods: Samples: Human osteochondral samples of variable degenerenerative state (n = 41, d = 8 mm, from four cadavers, and edges sealed using cyanoacrylate) were immersed in a dual CA mixture; cationic CA4+ (5 mg/ml, q = +4) and non-ionic gadoteridol (Prohance6), 10 mgGd/ml, q = 0). The Research Committee of the Northern Savo Hospital District (Kuopio University Hospital, Kuopio, Finland, ethical permission number: 134/2015) approved the sample collection. Measurements: Synchrotron µCT imaging was conducted before the dual CA immersion and at an early diffusion time point (2 hour) using two monochromatic X-ray energies at both sides of the iodine k-edge (32 and 34 keV). As reference parameters, optical density (PG content and distribution) of Safranin-O stained sections using digital densitometry and equilibrium Young’s modulus (Eeq, Hayes model7) using biomechanical indentation test were determined. In addition, Mankin score for each sample was determined from histological sections. Analysis: The non-contrast attenuation profiles were subtracted from the CA attenuation profiles of the samples. Next, depth-wise CA partition profiles were determined using Beer-Lambert law and Bragg’s additive rule for mixtures (Fig 2)8.

Statistics: Cartilage was divided into uniform layers of 10% thickness starting from articulating surface (0%) to cartilage-bone interface (100%) and partitions at different layers were correlated with the reference parameters. The relation between the CA partitions and reference parameters (PG content and Eeq) were evaluated with Spearman’s correlation coefficient (rs). The effectiveness of the normalization of the CA4+ partition with that of gadoteridol to improve correlation was tested according to Lenhard et al.9. The limit for statistical significance was set to p < 0.05.

Results: The dual contrast method enabled simultaneous determination of the depth-wise CA4+ and gadoteridol partitions within articular cartilage after 2 hours of diffusion (Fig 1). The normalization significantly improved the sensitivity to detect cartilage PG content and Eeq (mean |r| = 3.723, p = 0.033). Normalized CA4+ partition of the superficial and middle layers (0–10%, 10–30%, 30–60%, 60–90%, 90–100%) correlated significantly with PG content (0.497 < rs < 0.645), with Mankin score (r = -0.324 < rs < -0.330), and with Eeq (0.468 < rs < 0.748). Gadoteridol partition correlated negatively with Eeq (0–10%, 100–50%, 50–60%, 60–70%, 70–80%, 80–90%) and positively with Mankin score (0–10%, 10–60%, 60–70%, 0.324 < rs < 0.668).

Discussion: This is the first study describing dual-contrast synchrotron µCT imaging for characterization of human articular cartilage. By applying the monochromatic X-ray beam in high-resolution synchrotron µCT, the full potential of the dual-contrast technique was established. Significant correlations with the reference parameters prove that this technique permits the detection of changes in the structure, condition, and function (Fig 2 and 3) of cartilage already at a clinically feasible diffusion time point (2 hour). These results are in line with our earlier study using conventional µCT at a diffusion equilibrium8. In spite of the beam hardening related error associated with conventional CT systems, the dual-contrast method is not limited to CT systems with monochromatic X-ray beam.

Significance: This study provides the proof-of-concept of the dual-contrast technique in assessment of cartilage composition as imaging related uncertainties were minimized. The results support the translation of the method into clinical practice.


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