Dual-Contrast Agent Synergy in Computed Tomography Reveals Fibril-Reinforced Poroelastic Material Parameters of Cartilage

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INTRODUCTION: Finite element (FE) modeling can be used to simulate cartilage functional properties and to analyze the complex mechanical behavior of the tissue[1]. In addition, it enables researchers to gain insights into joint and tissue mechanics, providing valuable information for both basic research and clinical applications, such as designing interventions for joint disorders and optimizing treatment strategies. However, it is challenging to create subject-specific tissue models without invasive mechanical testing. Previously[2-4], we demonstrated the effectiveness of dual-contrast-enhanced computed tomography (CT) method in revealing cartilage elastic material parameters. In this imaging method, adjusting the concentration of a cationic contrast agent using a non-ionic agent, as a "normalization", amplifies the sensitivity beyond what is achievable with individual contrast agents[2]. Also, with the introduction of new Photon Counting Detector (PCD) technology the differentiation is achieved through a single scan. For the first time, we utilize the dual-contrast method and PCD-CT technology combination to study the suitability of the methodology to reveal more intricate material parameters within a comprehensive cartilage computational model. The model parameters correspond to constituent-specific functional properties of cartilage. While previous studies have demonstrated contrast agents' capacity to reveal these cartilage constituents[2,4], we hypothesize that the diffusion of suitable contrast agents directly reveals the constituent-specific functional parameters of the model.

METHODS: *Samples:* Fifteen distal intertrochlear groove (non-weight bearing) and medial femoral condyle (weight bearing) articular cartilage samples (cylindrical plugs, d = 8.5 mm) were harvested from healthy equine stifle joints (N = 15). *Biomechanics:* A four-step stress-relaxation protocol with a flat-ended cylindrical indenter (d = 0.55 mm) was utilized (Fig. 1 A). A step size was 4% of remaining cartilage thickness, ramp rate 100 %/s, and relaxation time 10 min. *FE model:* A sample-specific fibril-reinforced poroelastic FE model[1] was constructed using Abaqus (v6.12-3) to replicate the experimentally recorded loads. The replication was done by optimizing five sample-specific model parameters: initial fibril network modulus (E_0), strain-dependent fibril network modulus (E_{fe}), non-fibrillar matrix modulus (E_{nf}), initial permeability (k_0), and permeability strain-dependency factor (M). *PCD-CT:* A quarter of each sample was immersed in a mix of cationic tantalum oxide nanoparticles (Ta_2O_5-NPs, size ~3 nm, 20 mg·Ta_2O_5/mL) and neutral iodixanol (size ~1.5 nm, 40 mg·I/mL) bath for 96 h. The samples were then imaged in air with a custom built PCD-CT setup (voxel size 68 x 68 x 68 µm^3), using a voltage of 120 kVp (filtration of 3.0 mm Al and 0.5 mm Cu), and a current of 0.25 mA. For the spectral analysis, energy bins were restricted to 10-80 and 10-120 keV. *Analysis of CT images:* Contrast agent partitions (tissue attenuation divided by bath attenuation) inside the samples were calculated (Fig. 1 B) using a custom-made MATLAB (R2020b) code. A material decomposition, based on calibration measurements, was utilized to differentiate the two concentrations of the contrast agents inside the cartilage samples[5]. Normalized tantalum partition was calculated by dividing the measured tantalum partition with the iodixanol partition. *Statistics:* Linear correlation analysis (Spearman's rank correlation) was used to determine the relationships between optimized model parameters, and contrast agent partitions.

RESULTS: Normalized tantalum partition correlated positively with non-fibrillar matrix modulus (E_{nf}) and negatively with initial permeability (k_0) (Table 1), with the normalization influencing these correlations. Among the parameters, the strain-dependent fibril network modulus (E_{fe}) demonstrated correlation solely with individual iodixanol, while not exhibiting correlation with the normalized tantalum partition.

DISCUSSION: Our study demonstrates the dual-contrast CT method's ability to establish a unified sensitivity that captures the functional attributes of both the solid and fluid constituents of cartilage. The strongest association was found between normalized tantalum and non-fibrillar matrix modulus (R = 0.74). On the contrary, with iodixanol the association was the opposite (R = - 0.74). This can be attributed to differences in charge and size between the two agents. The larger and positively charged tantalum is attracted by negatively charged non-fibrillar matrix stiffness defining[1] proteoglycans (PGs), while the diffusion of smaller and neutral iodixanol is tied to the amount of free water and porosity. This is also consistent with the association of iodixanol and permeability of the present study (Table 1), because permeability increases the intake of iodixanol. Likewise, in previous studies the diffusion of conventional contrast agent molecules has been reported to be proportional to tissue permeability[6,7]. Thus, a synergistic sensitivity is found to reflect simultaneously the solid and fluid phases. Another interesting result is an inverse correlation between iodixanol and the strain-dependent fibril network modulus. This result is explained by the fact that the modulus describes the stiffening of the collagen network under compression, i.e., the denser structure in the deeper part of the cartilage, thereby impeding iodixanol diffusion.

SIGNIFICANCE/CLINICAL RELEVANCE: The dual-contrast CT method with photon-counting technology enables analysis of constituent-specific mechanical properties of cartilage. This methodology holds the promise of unveiling functional shifts linked to conditions like osteoarthritis, opening avenues for early detection and more targeted clinical interventions.

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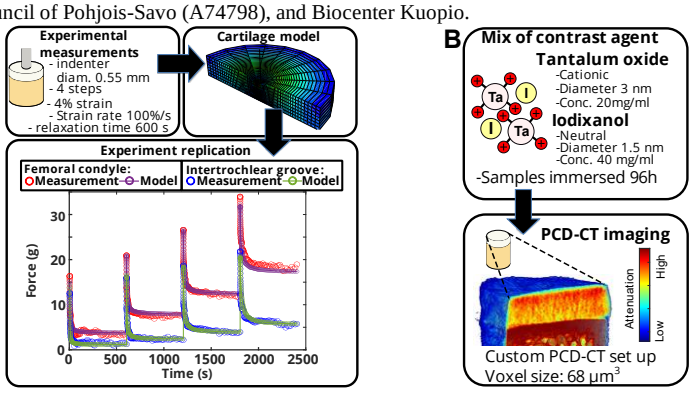

Fig. 1: For each sample A) a biomechanical cartilage model was created, and the experimental test was replicated to find sample-specific model parameters. B) Contrast-enhanced Photon-Counting Detector Computed Tomography (PCD-CT) was conducted and bulk partitions of tantalum and iodixanol were analyzed.

Table 1: Spearman's correlation coefficients between bulk cartilage contrast agent partitions and computational model material parameters. $p < 0.05$ *, $p < 0.01$**, $p < 0.001$ ***. Parameters presented in the table are initial fibril network modulus (E_0), strain-dependent fibril network modulus (E_{fe}), non-fibrillar matrix modulus (E_{nf}), initial permeability (k_0), and permeability strain-dependency factor (M).

Parameter	Normalized Tantalum	Tantalum	Iodixanol
E_0	0.18	0.13	- 0.15
E_{fe}	0.11	0.01	**- 0.44***
E_{nf}	**0.74*****	**0.64*****	**- 0.74*****
k_0	**- 0.42***	- 0.31	**0.45***
M	- 0.10	- 0.09	- 0.15