Contrast Enhanced CT Imaging of Cartilage: Effect of Matrix GAG Content

INTRODUCTION:
Magnetic Resonance Imaging1 and Computed Tomography2,3, using anionic contrast agents that act as mobile probes that are partitioned throughout the extracellular matrix (ECM) in inverse proportion to the fixed negative charge density of the proteoglycans, have been used to non-invasively quantify the GAG content of articular cartilage. The delayed gadolinium enhanced magnetic resonance imaging of cartilage (dGEMRIC) uses the anionic contrast agent gadolinium (Gd-DTPA) and Contrast Enhanced CT (CECT) uses iodinated anionic contrast agents, iothalamate or ioxaglate. Studies have confirmed that dGEMRIC can differentiate between healthy and arthritic cartilage in vitro and in vivo, however it has also been reported that the changes in the T1 relaxation time measured by dGEMRIC are affected by variations in the structure and composition of the cartilage other than GAGs. CECT is a promising technique that is less expensive, allows for faster image acquisition at higher resolution than dGEMRIC and can be used to image subchondral bone and cartilage simultaneously. CECT can quantify changes in the GAG content of articular cartilage. These studies assume that changes in the GAG content of the ECM is the primary factor affecting changes in the x-ray attenuation of cartilage evaluated by CECT. The aim of this study was to determine if changes in GAG content alone accounted for the changes in the x-ray attenuation of bovine osteochondral specimens measured by CECT where the GAG content was altered by enzymatic degradation.

METHODS:
Specimen Preparation: Thirty bovine osteochondral plugs (7mm diameter) extracted from the patella-femoral surfaces of five bovine knees were randomly assigned to one of three groups (n=10/group): normal control or one of two GAG depleted groups degraded by immersing the osteochondral plugs in chondroitinase ABC (Sigma-Aldrich) [0.1 U/mL in 50mM Tris, 60mM NaOAc, 0.02% BSA, pH 8.0] at room temperature for 8 or 30 hours. Contrast Enhanced Computed Tomography Imaging of Cartilage: Each osteochondral plug was immersed in 7 mL of an anionic, triiodinated contrast agent (Cysto Conray II, Mallinckrodt) for at least 12 hours to allow sufficient diffusion time for the contrast agent to partition itself throughout the ECM (based on separate diffusion study, data not shown). Four sequential, 10µm thick, transaxial pQCT images (Stratec, Germany) were obtained at 70µm in plane resolution and 1 mm inter-slice separation. Cartilage was segmented from bone using a semi-automatic contour based segmentation algorithm (AnalyzeTM, Mayo Clinic, MN). The mean x-ray attenuation of cartilage using the Hotplow Scale was obtained by averaging the attenuation values for the cartilage tissue over the four sequential transaxial CT images.

Biochemical assessment of GAG content: The articular cartilage was separated from the subchondral bone using a razor blade and the wet mass of the cartilage was obtained. The total GAG weight per mg wet weight of cartilage for each sample was calculated using the 1,9-dimethylmethylen blue (DMMB) colorimetric assay.

Statistical Analysis: Linear regression analysis (SPSS, Chicago) was used to express the x-ray attenuation measured by CECT as a function of the GAG content for each group of osteochondral plugs. The coefficient of determination was used to test the strength of each association. The slopes of the linear regressions fit to each group of osteochondral plugs progressively degraded by timed exposure to chondroitinase ABC were compared using the student t test to evaluate if changes in the slope of the relationship between x-ray attenuation and GAG content were affected by changes in GAG content alone.

RESULTS:
The change in the x-ray attenuation measured for each group of osteochondral plugs using CECT was linearly and inversely related to the GAG content of the articular cartilage: \( r^2 = 0.84 \) (control), \( r^2 = 0.85 \) (8 hour exposure to chondroitinase-ABC) and \( r^2 = 0.79 \) (30 hour exposure to chondroitinase-ABC), all \( p<0.0001 \). (Figure 1). Compared to the control group (slope = -19072), the slope (-24674) for the GAG depleted plugs exposed to chondroitinase-ABC for 8 hours was -22% higher (\( p = 0.244 \)) and the slope (-39557) for the GAG depleted plugs exposed to chondroitinase-ABC for 30 hours was -52% higher (\( p = 0.021 \)). The increase in x-ray attenuation was most evident in the superficial zone and less evident in the deep zone of the articular cartilage (Figure 2).

DISCUSSION:
The variation in x-ray attenuation measured by CECT reflected nearly 80% of the variation in the GAG content of the ECM for bovine osteochondral plugs progressively degraded by timed exposure to chondroitinase-ABC. However, the slopes of the regressions fit were different for each group and increased as a function of time of exposure to chondroitinase-ABC. Thus, the inverse relationship between GAG content and x-ray attenuation measured by CECT using an anionic contrast agent is non-linear (Figure 3). When the complete set of data is fit to a curve of the form, \( CECT \text{ attenuation} = A^*GAG \text{ content} + C \), the variation in x-ray attenuation measured by CECT reflects nearly 90% of the variation in the GAG content (Figure 3). If GAG was the primary factor that affected the x-ray attenuation measured by CECT, then the slopes of the regressions fit to each group of osteochondral plugs should be similar. The increase in the value of the slopes as a function of time of exposure to chondroitinase-ABC might be explained by an increase in the permeability of the contrast agent in cartilage caused by the concomitant loss of GAG from the matrix. Since the slope of the control group and the eight hour exposure group were not significantly different, there might be a minimum threshold of GAG depletion before the permeability of the cartilage is increased, as seen in late stage osteoarthritis. Further, it should be noted that chondroitinase-ABC is a GAG specific hydrolase that has minimal effect on collagen content. The loss of collagen along with GAGs might occur in diseased tissue may also affect the diffusion of the anionic contrast agents and accentuate the permeability effect. The work presented here demonstrates that the use of a linear relationship between GAG content and x-ray attenuation measured by CECT is inappropriate to represent the range of cartilage properties that might be encountered in health and disease, and that a non-linear relationship, such as the one presented here, may need to be derived for human articular cartilage that takes into account the effect of GAG content on the permeability of cartilage to the diffusion of anionic contrast agents into cartilage. Complete understanding of the physical and biochemical factors that affect diffusion of contrast agents into the cartilage will result in CECT of cartilage to become a viable clinical tool.

REFERENCES