INTRODUCTION
Osteoarthritis (OA) is a pathology characterized by degeneration and progressive erosion of the cartilage lining diarthrodial joints. While the etiology of OA is incompletely understood, proteoglycan (PG) depletion, followed by collagen loss, is part of its natural progression. Monitoring PG depletion is key to proper characterization of OA models (e.g. IL-1, collagen II antibody) and evaluation of therapeutic interventions. Advances in contrast-enhanced MRI, notably the dGEMRIC technique, have shown success in both detection of PG depletion and correlation of MRI relaxation constants with biochemical composition [1-3]. Microcomputed tomography (µCT) offers reduced scanning time over MRI at comparable spatial resolutions, but techniques have not yet been developed to allow µCT imaging of cartilage ultrastructure. This study evaluated the feasibility of a novel approach using contrast-enhanced microcomputed tomography to detect in vitro cartilage degeneration and to relate resultant changes in x-ray attenuation to changes in biochemical composition.

METHODS
Tissue Culture Forty full thickness cartilage explants (4mm diameter) were harvested aseptically from the patellar groove and femoral condyles of a 1-2 week old calf. After overnight culture in serum-free DMEM with antibiotic/antimycotic, explants were trimmed to a final thickness of 3 mm below the intact articular surface. To induce degradation, explants were cultured in serum-free DMEM ± 20ng/mL recombinant human interleukin-1α (IL-1α) for up to 10 days, with media changed and collected every 48 hours. Explants were removed at days 2, 4, 6, 8, and 10 (n=4/condition/time point). Microcomputed Tomography Immediately upon removal, explants (n=3/condition/time point) were immersed in Hexabrix™ (Mallinckrodt) for 2 hours at 37°C. Hexabrix™ is a tri-iodinated dimer that upon dissociation yields negatively charged, radioopaque ions. This approach exploits the negatively-charged dissociates, which are assumed to localize in a manner inversely proportional to the fixed charge density (FCD) of the cartilage matrix, primarily due to PGs. Using a Scanco VivaCT 40 at a 21µm voxel resolution, two explants were simultaneously scanned in an average of 10 minutes. After thresholding and 3D reconstruction, each cartilage explant was divided into 10 equal thickness subvolumes parallel to the articular surface. The mean x-ray attenuation was determined for all subvolumes of each sample. Biochemical Assays Following µCT imaging, explants were stored at -20°C in PBS with protease inhibitors. Subsequently, explants were sectioned using a sledge microtome into sections corresponding to the µCT subvolumes and analyzed for sGAG content using the DMMAB assay. Media were also assayed for sGAG concentration. Statistical Analysis Differences in sGAG release at each time point were evaluated using the Student’s t-test (p<0.05). Differences in attenuation between control and IL-1 groups at each time point were evaluated using a one-way ANOVA (p<0.05). Linear correlation was used to relate attenuation readings to sGAG content.

RESULTS
sGAG release profiles (Fig 1) are consistent with previously reported data [4] and illustrate significantly greater cumulative release from IL-1 treated samples beginning at day 6. No significant differences in attenuation between groups were noted at Days 2 and 4 (Fig 3). Importantly, significant differences in attenuation between the IL-1 and control groups were noted at days 6, 8, and 10, corresponding to significant differences in sGAG release. The "saddle-shaped" attenuation profiles (Fig 3) indicate variation in PG distribution through the explant depth, and the general shape of the attenuation curves is consistent between groups. A significant, negative Pearson coefficient (R = -0.591, p < 0.0005) was found between attenuation and subvolume sGAG content. The negative Pearson coefficient indicates that the contrast agent was in fact preferentially excluded from regions of higher sGAG content, supporting the assumed inverse distribution relative to FCD. Examination of evaluated attenuation gradient images (Fig 4) indicates that degradation generally progressed from the outer surfaces inward, although milder loss of PGs was noted throughout the sample interior.

DISCUSSION
This study demonstrates a novel application of µCT for high resolution imaging of the internal architecture of articular cartilage. Using an approach analogous to the dGEMRIC technique, this study illustrates the ability of contrast-enhanced µCT to detect cartilage degradation in an in vitro IL-1 stimulated explant model. While this study is preliminary a technique warrants optimization, the results not only indicate the potential of µCT to monitor cartilage degradation but also describe a significant, linear relationship between attenuation and sGAG content. The fast scan times of microcomputed tomography make it particularly attractive over MRI where scans of comparable resolutions and fields of view can run 2 hours. In addition, the introduction of µCT instruments capable of in vivo scanning at 10µm resolution offers intriguing possibilities for the noninvasive study of proteoglycan depletion and accumulation in OA animal models and for the evaluation of implanted tissue engineered constructs.

REFERENCES

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