Assessment of sGAG Content in Normal and Degraded Rat Articular Cartilage via EPIC-μCT

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Introduction: Proteoglycan (PG) loss is one of the earliest symptoms of osteoarthritis and usually occurs before gross morphological changes. Previously, measuring the Equilibrium Partitioning of an Ionic Contrast agent via microcomputed tomography (EPIC-μCT) was able to detect PG content and distribution in cultured bovine cartilage explants, providing high resolution spatial images of degeneration patterns (1). The principle of EPIC-μCT is that a negatively charged, radiopaque contrast agent will be preferentially excluded from regions of higher negative fixed charge density associated with higher PG content. This study further investigated whether EPIC-μCT was able to detect sulfated glycosaminoglycan (sGAG) changes in situ in rat articular cartilage during the maturation and aging process, after chondroitinase ABC digestion, and in an arthritis model.

Materials and Methods: Cartilage maturation and digestion: Male Wistar rats aged 4, 8, and 16 weeks (n=2 each) were sacrificed and both femora were harvested. The distal femur was incubated with 40% Hexabrix/60% PBS for 30 min at 37 degree and scanned with a μCT-40 (Scanco) at 45 kV, 177 mA, 200-ms integration time, and a voxel size of 16 μm (2). After scanning, right femurs were washed in PBS for 30 min to desorb the Hexabrix, digested with 1ml (0.1 U/ml) Chondroitinase ABC (Sigma) solution for 60 min at 37 degree, and then re-incubated with Hexabrix and rescanned. The 3D morphology of articular cartilage was produced, and the average attenuation (indicative of sGAG content) was determined. Following scanning, all femora were decalcified, embedded in GMA, cut at 8μm and stained for sGAGs with safranin-O. The optical density of red color of each section was calculated via Matlab, and sGAG content of each femur was determined by averaging values from the central section in both condyles. Arthritis model: Two male Wistar rats aged 8 weeks were anaesthetized and 1mg of monosodium iodoacetate (MIA) in 50μl saline was injected through the infrapatellar ligament of the right knee, with the left knee serving as a control. The rats were euthanized 1 week after MIA injection, and both femora and patellae were harvested, scanned and analyzed as before.

Results: Cartilage maturation: The average EPIC-μCT attenuation of the cartilage layer (Fig 1A) increased by 4.8% between 4 and 8 weeks of age (ANOVA, p<0.05) and further increased by 5.2% between 8 and 16 weeks of age (p<0.05), indicative of progressively decreasing sGAG density with age. Cartilage digestion The sGAG optical density in histological sections decreased by 41.9% after 1 hour of Chondroitinase ABC digestion (paired t-test, p<0.01, Fig 1C, Fig 2), indicating a decrease in sGAG content. Consistently, cartilage attenuation in EPIC-μCT images increased by 19.9% after digestion (paired t-test, p<0.01, Fig 1B, Fig 2), with a strong negative correlation (r=-0.86, p<0.01) between sGAG optical density and EPIC-μCT attenuation. MIA arthritis model: Consistent with early loss of sGAG, EPIC-μCT cartilage attenuation increased in the patellae of MIA injected joints (Fig 3) in both animals over 1 week post-injection, with an average attenuation 25.5% higher than in the control patellae.

Discussion: This study illustrates that EPIC-μCT imaging is able to detect changes in sGAG content in normal and degraded rat articular cartilage. In the aging study, EPIC-μCT imaging showed that sGAG content gradually decreases during cartilage maturation in the rat, consistent with previous histological reports. In the ex-vivo degradation study, both EPIC-μCT imaging and histology identified the lower sGAG contents in digested cartilage. The high correlation between these two methods suggested that EPIC-μCT imaging has high sensitivity to detect the changes in sGAG content. Moreover, although 2D attenuation maps are shown here, this novel technique provides full 3D spatial distribution of attenuation and thus has the potential to identify focal regions of degeneration in animal models. The preliminary study of the rat MIA arthritis model indicated that EPIC-μCT is indeed sensitive to the loss of sGAG content in early stages of experimental arthritis. In summary, these data demonstrate that EPIC-μCT has high spatial resolution and sensitivity to temporally assess changes in sGAG content, a critical factor for monitoring the progression and treatment of arthritis in small animal models. This novel imaging technique therefore provides a new high resolution approach to assess cartilage maturation and aging, degradation, and the effectiveness of pharmacologic therapies in small animal models.

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