Introduction: Evaluation of articular cartilage is a critical factor in the study and development of new disease modifying therapies for osteoarthritis (OA). Histology is traditionally used to evaluate articular cartilage morphology and the changes that accompany the progression of the disease. This, however, is both destructive and time consuming. A high resolution technique, like µCT, is a gold standard for bone microstructural analysis. However it is unable to detect soft tissues like cartilage due to its low x-ray attenuation. This is resolved by using a suitable contrast agent such as Hexabrix 320 (Ioxaglate), a negatively charged hexaiodinated dimer, which is resolved by using a suitable contrast agent such as Hexabrix 320 (Ioxaglate), a negatively charged hexaiodinated dimer, which compensates for the poor radio-opacity of proteoglycan (PG) containing soft tissues and has been used for imaging cartilage in bovines, rabbits and rats. Biological similarities to humans, small size, low cost, easy housing, availability and ease of creation of different strains and mutations make mice a popular model for biomedical research. It is however a challenge to image the extremely thin mouse femoral cartilage (~50 µm) as compared to the thick bovine cartilage or even rat cartilage (~130 to 400 µm). The earliest change that occurs with the onset of OA, much before the morphological changes, is the depletion of the PG content of the cartilage. The purpose of this present study was to determine if mouse articular cartilage could be visualized and its sulfated glycosaminoglycan (sGAG) content degradation monitored by applying the principle of EPIC- µCT.

Materials and Methods: Determination of equilibrium time: Intact soft tissue free femurs of twelve week old mice (n=3) were scanned using a µCT 40 (Scanco Medical, Switzerland) at 45 kVp, 177µA, 300ms integration time at 6µm resolution, in air. The femurs had been stored frozen. The distal ends were then incubated in 30% Hexabrix (gift of Mallinckrodt Inc, St. Louis, MO)/ 70% 0.15M PBS for 10, 25, 35 and 45 mins and scanned. The femora were placed horizontally in the vertical µCT scanning tube (diam: 12mm), to enable sagittal slices. The histograms of the x-ray attenuation values of the cartilage plateau at 30 minutes of incubation time is ideal for further experiments. Hexabrix enhanced visualization of cartilage (Fig.2) which enabled segmentation and isolation of the cartilage and the creation of 3D thickness maps for 30 minutes of incubation time is ideal for further experiments. Hexabrix enhanced visualization of cartilage (Fig.2) which enabled segmentation and isolation of the cartilage and the creation of 3D thickness maps (Fig.3).

Results: Equilibrium time: The average x-ray attenuation values of the cartilage increased with time and then reached a plateau (Fig.1). The plot showed that hexabrix reached equilibrium and hence 30 minutes of incubation time is ideal for further experiments. Hexabrix enhanced visualization of cartilage (Fig.2) which enabled segmentation and isolation of the cartilage and the creation of 3D thickness maps (Fig.3).

Discussion: This study illustrates that EPIC-µCT can be used successfully for imaging and detecting the sGAG content in murine knee joints. Incubation of the femurs in 30% hexabrix /70% PBS concentration for 30 minutes at 37°C prior to µCT imaging allows the cartilage to be segmented and isolated, enabling the quantification of articular cartilage morphology and composition. Further, the specificity of hexabrix contrasting of cartilage was confirmed by the finding that extensive digestion of the cartilage surfaces with chondroitinase ABC prior to hexabrix equilibration and scanning, led to a significant (~16 %) increase of cartilage attenuation. Using frozen samples as done in this work is a possible study limitation since one freeze- thaw cycle causes loss of PGs. Nevertheless, these data support the ability of µCT to image and detect changes in thin mouse articular cartilage. An application of this rapid and specific imaging tool to surgical and non-surgical models of murine OA will allow rapid and quantitative evaluation of cartilage loss or repair.


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