Quantitative Analysis of Mouse Articular Cartilage using Equilibrium Partitioning of Ionic Contrast Agent via Micro Computed Tomography (EPIC - µCT)

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INTRODUCTION

A qualitative and quantitative analysis of articular cartilage (AC) integrity is an important outcome measure for in vivo arthritis and cartilage repair experimentation. In our previous studies we used the EPIC-µCT approach to optimize methodological steps for murine cartilage imaging. This included contrast agent concentration and equilibration times, methods for tissue preservation (formalin fixation or rapid freeze in PBS with protease inhibitors (PIs)).[1] In the present study, we have applied this method for quantitative analysis of femoral cartilage in C57B/6 male mice of different ages. We have determined thickness, volume, and average attenuation values (which are a measure of sulfated glycosaminoglycan (sGAG) content of the AC [2]) in 6, 10 and 14 week old mice. In addition, we show that the method is also capable of detecting a variety of lesions, such as fissures and surface abrasion.

MATERIALS AND METHODS

Age study: Intact femurs carefully dissected clean of adherent soft tissue from C57B/6 mice at 6 weeks (n=6), 10 weeks (n=6) and 14 weeks (n=9) were incubated in 15% hexabrix (gift of Mallinckrodt. Inc, St. Louis, MO)/85% PBS and PIs for 30 minutes and scanned in air using a µCT 40 (Scanco Medical, Switzerland) at 45kVp, 177µA, 300ms integration time at 6µm resolution. The cartilage and subchondral bone were manually segmented and grey scale frequency distributions used to determine average attenuation values for the cartilage. Using these values, scanner software was used to further segment the cartilage and obtain its thickness and volume. Following scanning, specimen were fixed in formalin, decalcified, sectioned and stained with Safranin O to determined cartilage thickness using OsteoMeasure software (OsteoMetrics, Inc.). Here the tidemark was used as an indication of cartilage depth and thickness was measured accordingly. Analyses of variance with Bonferroni post-hoc comparisons were used to assess aging effects, while t-tests were used to compare histology and EPIC-µCT measurements of cartilage thickness. Lesion Study: Using a scalpel, a longitudinal (sagittal) cut was made in the cartilage of a 6 week old mouse femur on the lateral condyle and cartilage partially shaved off the surface of the medial condyle prior to scanning as described above.

RESULTS

Age study: There were significant age effects for thickness (ANOVA $p < 0.001$) and volume (ANOVA $p < 0.001$). Specifically, compared to the 6 week group, there was a 23% reduction in thickness at 10 weeks and a 27% reduction at 14 weeks (Fig.1a). Compared to the 6 week group, there was a 37% reduction in volume at 10 weeks and a 43% reduction at 14 weeks (Fig. 1b). The decrease in cartilage thickness in the older age groups is also evident from the thickness maps (Fig. 2).

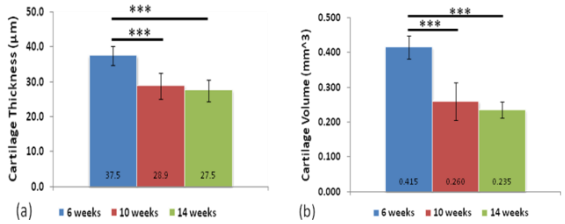

Fig.1: The age study showed significant differences in the (a) average thickness and (b) cartilage volume between the 6 and the 10 as well as the 6 and the 14 week age groups (Data represents mean values and error bars represent SDs, *** indicate $p < 0.001$).

Compared to 6 weeks, the average attenuation values were 14.5% higher ($p=0.01$) at 10 weeks and 11.7% higher at 14 weeks ($p = 0.03$) (Fig.3).

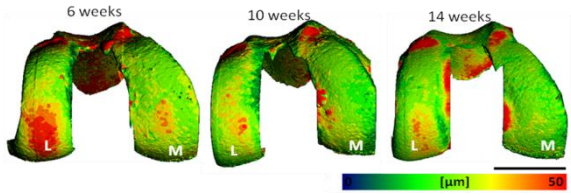

Fig.2: Thickness maps of 6, 10 and 14 week old mouse cartilage show the decrease in thickness between the 6 and the 10 and 14 week age groups. The thickest regions are indicated by red. The scale bar indicates 1mm.M= Medial condyle and L = Lateral Condyle.

The µCT values for thickness of the 10 week age group were compared with those obtained from histology (Fig.4) and they were not different ($p = 0.287$).

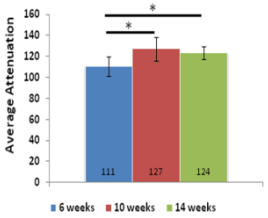

Fig.3: The increased average attenuation of the 10 and 14 week age groups may indicate a reduction in PG content (Data represents mean values and error bars represent SDs,* indicates $p<0.05$).

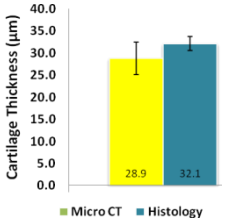

Fig.4: Comparison between the thickness measurements of 10 week age group taken using µCT and histology (Data represents mean values and error bars represent SDs).

Lesion Study: The lesions created on the condylar surfaces of the mouse cartilage are easily visualized (Fig.5).

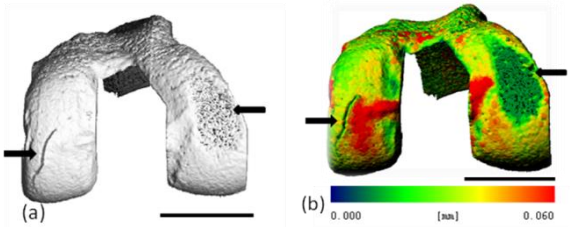

Fig.5: The induced lesions can be seen on the condyles. The figure (a) is the topographical map while (b) is the thickness map of the 6 week old mouse cartilage. Black arrows indicate the lesions. Scale bar represents 1mm.

DISCUSSION

Quantitative analysis of the thickness and volume of the cartilage showed statistical differences between the young and the older mice. A similar trend was seen in a rat study.[3] Further, good agreement of cartilage thickness values was found between the histology and µCT data. In mice, the µCT imaging could be considered as "true cartilage" imaging as compared to "cartilage and calcified cartilage" imaging since the contrast agent is seen to highlight only the cartilage above the tidemark, as opposed to inclusion of calcified cartilage in histology that Safranin O and Toludine Blue stains are unable to differentiate. The increase in attenuation may be representative of a decrease in PG content in the articular cartilage of the older mice. The present study offers further evidence that µCT provides a new means of viewing and quantifying changes in AC morphology and PG content in mouse models.

References: [1] Kotwal, N et al, ORS 2010, Poster 1372; [2] Palmer, A et al, (2006), PNAS, 103(51):19255-260; [3] Xie, L et al, (2008), Osteoarthritis Cartilage, 17(3):313-20

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