Imaging the Effects of Trauma on Human Articular Cartilage using Micro Computed Tomography (µCT)

Introduction: Articular cartilage covers the surface of all diarthrodial joints and is responsible for smooth articulation. This tissue is susceptible to injury from either acute or chronic repetitive trauma and nearly one million Americans are diagnosed with chondral lesions each year. Having an effect on such a large cohort has stimulated efforts to preserve joint integrity using surgical and non-surgical techniques. In doing so, evaluation of articular cartilage post preservative, reparative and/or restorative therapies and/or treatment is crucial. Traditionally, histological analyses have been employed to evaluate cartilage morphology both pre and post treatment to evaluate changes, however histology is both destructive and time consuming.

Previous work used µCT to evaluate cartilage morphology and proteoglycan content. 1,2 In order to visualize the articular lining, contrast agent Hexabrix 320 (Ioxaglate) was used. This agent presents with a negatively charged iodinated dimer which correlates inversely to the proteoglycan (PG) content of the lining soft tissue. GAG chains are long unbranched polysaccharides consisting of repeating disaccharide unit; with chondroitin sulfate being the most prevalent. GAG chains carry a negative charge and subsequently repel negatively charged iodinated contrast dye. Therefore, by examining attenuation values the concentration of GAG chains can be inferred while maintaining detailed information about their localization. This technique has previously been used for imaging cartilage in bovine, rats and mice, while this study adopts the same principles to human articular cartilage. 1,3

The purpose of this study was to use hexabrix in order determine if changes in articular surface following trauma and treatment can be monitored using this non-destructive technique and whether the results obtained with this method correlate with those of histology and cell viability.

Materials and Methods: Articular cartilage impaction: Grade 0 donor human tali procured from Gift of Hope (Elmhurst, IL) were impacted with a 4mm indenter using an impulse of 1Ns, generating a peak force of 600N. 8 mm cartilage plugs containing the 4 mm impacted core and 4mm adjacent ring were removed and randomized to four groups, i) control; ii) impacted; iii) P188 (8ug/ml) treatment; and iv) OP (100ng/ml) treatment. Each group was cultured in serum free Dulbecco’s modified Eagle medium supplement with 100U penicillin and 100 µg/ml gentamicin at 37°C and 5% CO₂ atmosphere for a maximum of 14 days. P188 or OP-1 were added at day 0 and were kept for 48 hours. After culture, the tissue explants were formalin fixed for 24 hours. Following fixation, each explant was submerged in 100U penicillin and 100 µg/ml gentamicin at 37°C and 5% CO₂ atmosphere for a maximum of 4 days. P188 or OP-1 were added and kept for 48 hours.

Results: Following culture and scanning at various time points, x-ray attenuation values increased with impaction (Fig. 1, impacted panel, images from left to right). There were clefts within the reconstructed images in the impacted specimens with the highest attenuation signal in close proximity, implying localized loss of glycosaminoglycan (GAG) chains. In addition, a depth-dependent change in attenuation was also observed at varying depths. (Fig. 2).

Culturing in the presence of P188 or OP-1 largely abrogated the impaction-induced increases in attenuation values. Interestingly, an impaction-induced cleft was found, but was associated with only a minimal change in attenuation values.

Discussion: This study illustrates, implying localized loss of glycosaminoglycan (GAG) chains. In addition, a depth-dependent change in attenuation was also observed at varying depths. (Fig. 2).

Fig 1: µCT images of cartilage explants cultured for 14 days at varying depths.

Fig 2: Attenuation levels at varying depths.