Implications of Trauma and Motion on the Synthesis of Proteoglycan-4 in Adult Human Ankle Cartilage Explants

INTRODUCTION:
Proteoglycan-4 (PRG4), also known as lubricin and superficial zone protein [1], is a functional boundary lubricant that also inhibits abnormal protein deposition and prevents cellular adhesion [2]. The mechanosensitive expression of PRG4 [3] is altered by damage to articular cartilage [4]. A recent investigation of articular cartilage from the femoropatellar groove of young bovine calves showed an upregulation of PRG4 mRNA, immediately after damage [5]. It has also been shown that motion acts as a biomechanical stimulus to upregulate PRG4 synthesis in healthy bovine cartilage explants [3,6] and in chondrocyte seeded scaffolds [7]. Although the presence and synthesis of PRG4 has been investigated for human articular cartilage [8], the influence of injury remains unclear, as does the role of articular motion following injury [5]. The purpose of this study is to investigate the implications of damage and articulation, on the expression of PRG4 in adult human talar cartilage explants.

MATERIALS AND METHODS:
Adult human cartilage was obtained from ankle talus pairs of tissue donors (4M/2F aged 80 ± 7 years), within 24 hours of death through the Gift of Hope Organ and Tissue Donor Network (Elmhurst, IL). Only normal cartilage (Collins grade 0) without prior fibrillation/erosion was chosen for the study. Four circles (φ 10 mm) were outlined on the trochlear surface of each intact talus and randomized into two ‘damaged’ and two ‘undamaged’ groups. All explants from one ‘damaged’ and one ‘undamaged’ group were subjected to articulation (= ‘motion’ groups) and explants from the other two groups were rested through the duration of testing. On day 0, damage was initiated centrally within the circles using the flat end of a cylindrical indenter (φ 4 mm) that was accelerated onto the cartilage surface by a pneumatically controlled impactor [9]. A single impact had a momentum of 1 Ns and generated a peak contact force of up to 600 N, initiating (partial) damage to the cartilage surface. Full thickness cartilage explant discs (4 per ankle) were then surgically removed from the circular outlines and organized into their previously assigned four groups. Each explant was individually cultured at 37°C, 5% CO2, and high humidity, in 2 ml of DMEM-F12 medium, of which 1 ml was collected and replaced daily. On days 1 to 5, explants from the damaged and the undamaged motion groups were daily subjected to two 1-hour long articulation cycles using a motion simulator [9]. Articulation was provided by pressing a ceramic ball against the explant surface and then oscillating it in a sine-wave at 1Hz ± 30°. The compression at the ball apex was 10% of explant thickness. The ball was moved ±2.5 mm over the explant surface in a curvilinear manner at 0.1 Hz. At days 0 and 6, the histopathological grades (OARSI score) and chondrocyte viability of the explants were determined. PRG4 content in the collected medium was quantified using ELISA assay. Statistical analyses were performed using one- and two-way ANOVA. Data are reported as mean ± SEM. The level of significance was set to 0.05.

RESULTS:
Initial Response: As expected, the histopathological grades were higher for damaged explants (2.4 ± 0.24 vs. 0.6 ± 0.24; p<0.001). Viability of the superficial zone of damaged cartilage explants was significantly reduced when compared to undamaged cartilage (51.4 ± 9.9 vs. 81.0 ± 5.1 %live, p<0.03). Viability for the total explant, however, remained similar (75.7 ± 5.5 vs. 88.5 ± 4.2 %live, p=0.05). Analysis of the biosynthetic activity demonstrated that trauma to the surface resulted in a significant PRG 4 increase (2.2 ± 0.4 vs. 1.5 ± 0.3 µg/ml, p<0.04). Normalizing the PRG4 synthesis by living cells further enhanced this difference as shown in Figure 1A.

Day 6 Response: Histopathological analysis demonstrated that damaged explants sustained their higher grades when compared with their undamaged counterparts (p=0.04). Articulation had no influence on the grades. Analysis of the superficial zone using two-way ANOVA verified that damage significantly decreased (p=0.02) chondrocyte viability, while articulation demonstrated no influence (p=0.62) on viability. There was no interaction between these two factors (p=0.95).