Introduction. Extensive research is underway to develop more effective and conservative therapies to halt or slow the degeneration of cartilage that occurs in osteoarthritis (OA) such as cytokine antagonists, anti-proteases, and growth factor agonists (1). In order to utilize and evaluate these novel approaches the early diagnosis of OA is critical as is the monitoring of the therapies used. The use of magnetic resonance (MR) modalities for cartilage imaging is well accepted (2,3) and the utility of MR to assess cartilage biochemistry is under investigation (4-6). The objective of this study was to test the hypothesis that magnetic resonance microscopy imaging (MRI) of engineered cartilage correlates with the biochemical composition and biomechanical properties of neocartilage, and that MRI can be used to evaluate cartilage matrix material properties during focal tissue development and degradation. We utilized a hollow fiber bioreactor system in which neocartilage is formed from isolated chondrocytes in a highly reproducible and focal manner (4,5,7).

Methods. Chondrocytes from Day 16 embryonic chick sterna were inoculated into a hollow fiber bioreactor and allowed to grow for up to 4 weeks with and without exposure to chondroitinase ABC. The negative fixed charged density (FCD) of the cartilage was measured using a modification of the gadolinium (Gd) exclusion method (8). In addition, the water, proteoglycan, collagen and DNA contents were determined together with the dynamic and equilibrium moduli of the neocartilage. The effect of maturation time (age) on the biochemical, MRI, and biomechanical properties were analyzed by ANOVA. Tukey/Kramer post-hoc tests were used to determine statistical differences between treatment groups. The effects of chondroitinase ABC treatment on cartilage properties were analyzed using Student's (two-tailed) t-tests. The correlations between MRI measurements, biochemical composition, and biomechanical properties were analyzed by linear regression using Systat software. Significance level was set at 0.05.

Results. Specific biochemical and biomechanical properties of the neocartilage such as chondroitin sulfate content (Fig. 1, P<0.0009), tissue cross sectional area (Fig. 2, P<0.008), and equilibrium modulus (Fig. 3, P=0.037) varied as a function of maturation. The negative fixed charge density Fig. 4) showed an increase with time but was not statistically significant while the collagen (hydroxyproline content) did not vary.

Exposure of the neocartilage to chondroitinase ABC resulted in alterations in the properties of the neocartilage. The Gd-enhanced MR image indicated a lower negative FCD in the 4-week-old chondroitinase-treated tissue compared to control. In addition, the cartilage volume within the bioreactor (cross sectional area) was less in the chondroitinase-treated tissue compared to control. Statistical comparisons revealed significant decreases in the negative FCD (P<0.02), proteoglycan content (P<0.01), as well as the dynamic and equilibrium moduli (P<0.001) as a result of the chondroitinase treatment. These results support the hypothesis that non-invasive assessment of neocartilage matrix provides information regarding the specific biochemical and biomechanical properties of the tissue.

Discussion. The present study addresses the hypothesis that MR analysis of neocartilage developing in a hollow fiber bioreactor will provide an estimate of the fixed charge density of matrix that correlates with the biochemical and biomechanical properties of the tissue. The use of the hollow fiber bioreactor model system is particularly relevant to the study of both normal and diseased articular cartilage since the tissue that forms is typical hyaline cartilage with abundant type II collagen mRNA expression (4,5,7). The neocartilage that forms is also focal in nature which models the situation that might occur in early OA lesions or in reparative cartilage resulting from transplantation (9,10). The chondroitinase treatment produced a cartilage matrix with reduced chondroitin sulfate compared to the control using direct biochemical measurement. Also, the treated cartilage was softer than control tissue with a reduction in both the dynamic and equilibrium moduli. The average FCD of the treated cartilage as determined by MRI was less than 50% of the control value which correlated well with the biochemical and biomechanical endpoints. These results were confirmed by demonstrating linear correlations between the matrix FCD and the biomechanical properties of the tissue as well as between the biomechanical properties and the chondroitin sulfate content of the cartilage. These results represent a strong demonstration that non-invasive MR imaging of neocartilage detects a change in the FCD that correlates with a change in the tissue biomechanical properties. Recently it has been shown, using delayed gadolinium-enhanced magnetic resonance imaging of cartilage, that T1 reflects the glycosaminoglycan concentration in autologous chondrocyte transplantation in human patients (11). The data presented here extend the correlations to include biochemical and biomechanical endpoints and are even more significant considering the likelihood that this approach will be used routinely in the clinic.

References
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