Introduction: Osteoarthritis (OA) affects over 40 million people in the United States and is the source of significant pain and disability. The knee joint has significant higher incidence of primary OA compared with the ankle joint, suggesting that innate differences in the articular cartilage of these joints may contribute to the onset or progression of disease (1). For example, pro-inflammatory cytokines such as interleukin 1 (IL-1) have been implicated in the pathogenesis of OA and suppress proteoglycan synthesis significantly more in knee than ankle chondrocytes (2). Furthermore, ankle cartilage has a lower water content, higher sulfated glycosaminoglycan content, higher equilibrium modulus, and higher dynamic stiffness than knee cartilage (3). These differences suggest that the decreased response to IL-1 observed in ankle chondrocytes is either secondary to innate cellular behavior or a result of exposure to a lower concentration of inflammatory molecules secondary to the denser extracellular matrix.

Articular cartilage is an avascular, aneural tissue that depends predominantly upon diffusion for transport of nutrients, metabolic wastes, signaling molecules and inflammatory mediators. Cartilage is divided into three major structural zones: superficial, middle, and deep, and the structure of these zones influences solute transport. Medium-sized molecules, such as 40 and 70 kDa dextrans, diffuse more slowly in the superficial zone than in the middle and deep zones (4). Studies have also shown that diffusivity is dependent upon proteoglycan content (5,6). The diffusion coefficient is a measure of solute transport, as measured by the diffusion coefficient and partition coefficient. The diffusion coefficient is a measure of the rate of diffusive transport of a molecule through a substance, while the partition coefficient measures the relative amount of a molecule that can permeate the substance. Furthermore, we investigated whether these differences were zone-dependent.

Methods: Cartilage samples were obtained from intact frozen cadaveric ankle and knee joints. Normal cartilage was graded using a modified Collins score and only specimens of grade 0-2 were used (7). Full-thickness cartilage explants harvested from the lateral dome of the talus and medial femoral condyle were placed in solutions containing fluorescein-conjugated dextrans of 70 kDa molecular weight at 0.032 mM in phosphate buffer solution and allowed to equilibrate at 4°C for 72 hours. The diffusion coefficient was measured using fluorescence recovery after photobleaching (FRAP) as previously described (4).

For partition coefficient measurements, full-thickness 4 mm punch biopsies were obtained from the lateral dome of the talus and medial femoral condyle. The samples were placed in a 0.60 mM solution of 70 kDa dextran for 24 hours and then transferred to a solution of phosphate buffered saline (PBS). After an additional 24 hours the concentration of fluorescein dextran in PBS was determined using a GENios Multi-Detection Reader (Tecan) at 535 nm emission. The partition coefficient was calculated as previously described based on the water content of the sample (8).

The superficial zone was manually dissected from the remaining cartilage. The cartilage was then sectioned into middle and deep zones using a Lancer series 1000 vibrotome. The samples were digested in 450 µl of 125 µg/mL papain at 64°C for 48 hours. Proteoglycan, collagen and DNA content of the digested cartilage specimens were determined using the dimethylmethylene blue (DMB), hydroxy-proline, and PicoGreen assays, respectively. The results were analyzed using ANOVA with Tukey HSD post-hoc tests and unpaired t-test.

Results: No differences were observed in the diffusion coefficients between ankle and knee cartilage (Figure 1a). The mean diffusion coefficients for ankle and knee cartilage were 34.6 and 35.4 µm²/s, respectively (N=9 ankle, 6 knee). The partition coefficient was significantly lower in the ankle than in the knee (Figure 1b). Average partition coefficients for ankle and knee cartilage were 0.0103 and 0.019, respectively (N=10 ankle, 8 knee). Total proteoglycan content was significantly higher in the ankle than in the knee (Figure 1c). PG content was also significantly higher in the middle and deep zones compared to the superficial zone in both the ankle and knee (N=10 ankle, 8 knee). The PG content per wet weight and the partition coefficient showed a significant inverse relationship (p<0.0001, R²=0.36). Water content showed a trend toward being lower in the ankle than in the knee with average values of 72.5% and 75.2%, respectively (p=0.06). Collagen content, as measured by hydroxy-proline, showed no significant difference between ankle and knee, but was higher in the superficial than middle zone (Figure 1d).

Discussion: The findings of this study support the hypothesis that different structural properties of ankle and knee cartilage are associated with differences in solute transport. The partition coefficient of 70 kDa dextran was 47% lower in the ankle than in the knee. This difference in partition may, in part, explain the decreased response of cartilage in the ankle joint to IL-1, as compared to the knee joint (2), by decreasing the effective interstitial concentration of this molecule for the same concentration in the external bathing solution. However, no significant differences were observed between the diffusion coefficients in ankle and knee cartilage.

The higher PG content in the ankle and the lack of differences in collagen content between ankle and knee cartilage are consistent with results reported by Treppo et al (3). The zonal differences in PG content are consistent with Maroudas who showed lowest levels of PG in the superficial zone (6). The higher PG content and lower water content suggest that ankle cartilage has a smaller effective pore size than knee cartilage, which would be consistent with a lower partition coefficient. The diffusion coefficient, however, does not appear to be correlated to the tissue PG content. These findings suggest that the compositional differences between ankle and knee cartilage contribute to a difference in partition coefficient, and support the hypothesis that differences in transport properties between cartilage of the knee and ankle may play a role in the differences in the incidence of OA in these joints.


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