CARTILAGE MECHANICS DETERMINED WITH OSMOTIC LOADING VARIES BETWEEN WEIGHT-BEARING REGIONS IN THE MOUSE KNEE

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Introduction. Mechanical properties of cartilage have been shown to vary between sites of high and low weight-bearing within the knee joint. For example, the tensile moduli of cartilage from low weight-bearing regions of the femoral condyle were shown to be stiffer than femoral cartilage from high weight-bearing regions in the human knee joint (Akizuki et al. 1986). Collagen content and organization are important determinants of cartilage mechanics in tension, and these differences were presumed to be related to the higher collagen to proteoglycan ratio and the degree of collagen organization in the low weight-bearing areas of the distal femur. In small animal joints, a similar understanding of relationships between cartilage mechanics, composition, structure and functional load-bearing has been limited by the difficulties associated with handling small tissue samples. An osmotic loading method was recently developed to quantify the mechanical properties and negative fixed charge density of cartilage in small animal joints (Flahiff et al. 2002a). In this study, this osmotic loading method was adapted to the study of mouse diarthrodial joints and used to study regional variations in cartilage mechanics and their relationship to functional load-bearing in the mouse knee.

Methods. Cartilage mechanics and proteoglycan-associated fixed charge density were determined at the tibial plateau of mouse knee joints. On the tibial plateau, the peripheral sites covered by the meniscus (COV) have been shown to transmit substantial loads across the joint surface and so may be considered to be high weight-bearing areas. The central-most cartilage sites not covered by the meniscus (UNC) are believed to play a lesser role in load-bearing across the knee joint. Tibiae (n=7) were obtained from 6 month-old mice (Balb-6), and planar cartilage-bone samples prepared from the medial tibial plateau by microtoming (Figure 1). Samples were fluorescently labeled (acridine orange, LDS, Molecular Probes) to stain cell and bone nuclei as markers for strain calculation. Samples were equilibrated in a hypotonic saline solution (0.015M NaCl, 30 minutes) and imaged under free-swelling conditions using confocal laser scanning microscopy. Samples were then equilibrated in a hypertonic solution (2M NaCl) to eliminate contributions to matrix swelling from the glycosaminoglycans (reference state), and images were again obtained. Swelling-induced strains were calculated with respect to the hypertonic state throughout the imaged region, as described previously (Flahiff et al. 2002a, Narmoneva et al. 1999).

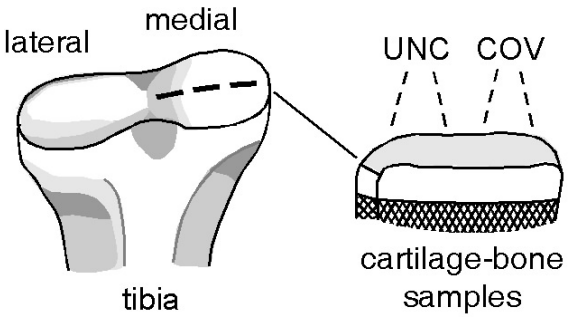

Figure 1. Schematic showing site of sample harvesting on the medial tibial plateau, with cartilage areas designated as covered (COV) or not covered (UNC) by the meniscus.

A semi-quantitative histochemical analysis was performed to determine the negative fixed charge density of the cartilage sections. This method is based on the assumption that the negatively-charged glycosaminoglycans within articular cartilage linearly contribute to a red intensity when stained with safranin-O, as shown previously (Martin et al. 1999, Flahiff et al. 2002a). Digital images of stained, cartilage sections (5 μm, safranin O/fast green) were analyzed to determine red intensity throughout the imaged region. Adjusted red content values were converted to reference fixed charge density using a previously determined calibration constant (Flahiff et al. 2002a).

To determine the mechanical properties, a homogeneous triphasic model for cartilage swelling was used to predict the uniaxial modulus from depth-dependent measures of the swelling strains and fixed charge density (Lai et al. 1991, Narmoneva et al. 2002). In prior work,

the uniaxial moduli determined by this osmotic loading method compared favorably to tensile moduli determined from conventional tensile testing of site-matched tissues (Narmoneva et al. 2001). For all studies, parameters obtained for the full-thickness cartilage layer were divided into two areas (Figure 1) – the central-most region of the medial tibial plateau that is not covered by the meniscus (UNC), and the peripheral region covered by the meniscus (COV). Paired t-tests were used to test for differences in measures of the uniaxial modulus, thickness-averaged swelling strains, and thickness-averaged fixed charge density between UNC and COV regions of each sample.

Results. Swelling strains were significantly higher in cartilage from the uncovered region of the tibial plateau, compared to the covered regions ($p=0.01$). The uniaxial modulus was significantly lower in the uncovered cartilage compared to the covered regions ($p=0.02$, Figure 2), with an average pair-wise decrease of 44%. There was no difference in thickness-averaged fixed charge density between the two regions with an average of 0.081 mEq/ml tissue water for all samples ($p>0.05$).

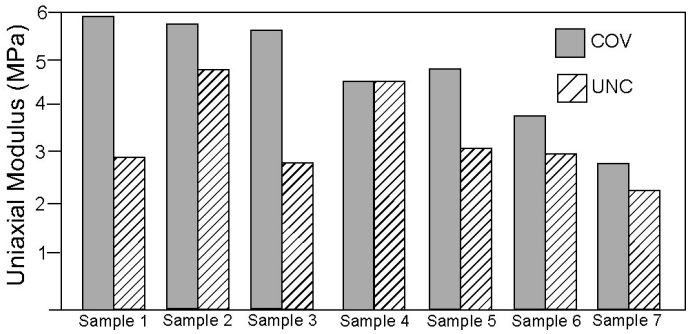

Figure 2. Moduli (MPa) for the covered and uncovered regions of the tibial plateau in paired specimens.

Discussion. Previously, the osmotic loading method was used to quantify region-averaged mechanical properties of mouse knee cartilage, for comparison against cartilage from mice with a collagen defect (Flahiff et al. 2002b). The results of this study present a first attempt to identify region-specific cartilage stiffness in the mouse knee joint, with findings of significant differences in the uniaxial modulus of tibial cartilage amongst regions. Cartilage on the tibial plateau at sites that are not covered by the meniscus was found to have a lower uniaxial modulus. This site was previously shown to have a more highly disorganized collagen fibrillar network in most species (Clark 1991). The findings of lower values for moduli in the uncovered cartilage are consistent with the more disorganized collagen fibrillar network, since collagen composition and structure are known to be important determinants of soft tissue mechanics in tension. This finding is further suggested by prior studies that have demonstrated the uniaxial modulus to reflect cartilage stiffness in tension.

The uncovered cartilage sites on the tibial plateau are not believed to contribute as significantly to load support and transmission as the covered sites in the knee joint and thus may be considered to be the lower weight-bearing regions. In an early study, Akizuki and co-workers (1986) reported lower tensile moduli in the high weight-bearing regions of the patellar groove and femoral condyle of human knee joints, a finding that they attributed to a lower ratio of collagen to proteoglycan contents compared to low weight-bearing regions. In that study, the tensile properties were determined in cartilage strips oriented parallel to the cartilage surface. However, the modulus obtained here from the osmotic loading method reflects contributions from the collagen fibrillar network that resist swelling in a direction perpendicular to the cartilage surface. This difference in testing protocol, as well as joint surface, may partly explain the conclusion of the current study, that cartilage stiffness is greater in the area of higher collagen content, and why that differs from the earlier study of human cartilage. Nevertheless, the results of both studies are consistent in pointing to a relation between collagen composition, structure and mechanics in tension, and suggest that collagen organization, and potentially content, may be greater at the areas not covered by the meniscus on the tibial plateau in the mouse knee joint.

In summary, the findings of the current study demonstrate that the structure-function relations for cartilage in the mouse knee joint are not unlike that of the human, in that organization of the collagen fibrillar network varies with load-bearing requirements in a region-specific manner and contributes significantly to cartilage mechanics in tension.

Acknowledgments. Supported with funds from NIH (AR45644). The authors wish to thank Steve Johnson for technical assistance.

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