Introduction: Cartilage swelling is a well-documented and early change in articular cartilage with osteoarthritis (1). In previous studies, we demonstrated that quantification of cartilage swelling strains in an osmotic loading test may be used to depict degeneration in a canine model of osteoarthritis (2). We further demonstrated that this technique may be used to quantify cartilage mechanical properties (3), and is particularly useful for studies of small animal joints as it obviates the need to contact or grip the tissue sample. Spontaneous development of osteoarthritis in the guinea pig has been well characterized, with histological evidence of degeneration in the tibial plateau between 7 and 12 months of age. This model mimics many histological features of osteoarthritis in the human and thus is attractive for studying the etiology of human osteoarthritis. However, no corresponding information on cartilage mechanics in the guinea pig is available due to the small joint size. In this study, we utilized the osmotic loading technique in two age groups to quantify cartilage matrix properties of the medial tibial compartment, the site of onset of osteoarthritis in the guinea pig.

Methods: Tibiae were harvested from young (2 month, n=6) and retired breeder (12 month, n=6) male Hartley guinea pigs. Since determination of cartilage matrix properties requires both cartilage swelling strains and physicochemical properties (e.g., fixed charge density, c0), adjacent slices perpendicular to the cartilage surface were prepared from the medial tibial plateau of the left tibia for both swelling strain and biochemical analyses. For quantification of swelling strains, samples were fluorescently labeled to identify cartilage and bone cell nuclei as markers (2). Swelling strains were quantified relative to a zero-strain, hypertonic reference state (2M NaCl). Thus, samples were first equilibrated for 4h in the reference solution and viewed using a confocal laser scanning microscope. A high resolution digital image was recorded as the reference image (Fig. 1). Slices were then equilibrated 4h in a hypotonic solution (0.015M NaCl) and a second image recorded. Bone and cartilage markers were identified on the reference and equilibrated 4h images to identify cartilage and bone cell nuclei as markers (2). Swelling strains were quantified from the image pairs and the strains were calculated from the measured strains.

For quantification of fixed charge density, adjacent slices were stained with safranin-O, and densitometric measures of an adjusted red content were obtained from digital images, in a modification of a method described previously (5). Intensity of safranin-O staining was converted to c0 (mEq/ml tissue water) using a calibration coefficient of 0.0032mEq/ml water at 2M NaCl. Thus, samples were first equilibrated for 4h in the reference solution and viewed using a confocal laser scanning microscope. A high resolution digital image was recorded as the reference image (Fig. 1). Slices were then equilibrated 4h in a hypotonic solution (0.015M NaCl) and a second image recorded. Bone and cartilage markers were identified on the reference and swollen images, and the planar components of swelling strain were calculated relative to a zero-strain, hypertonic reference state (2M NaCl).

Discussion: The results of this study provide the first data for the mechanical properties of degenerated guinea pig cartilage. In previous work, the moduli determined from the osmotic loading test were found to compare favorably with those of cartilage in tension, so the moduli reported here likely reflect the tensile stiffness of the cartilage solid network. In this study, the moduli of older cartilage were higher, indicative of stiffer cartilage. This finding suggests that changes due to aging predominate between these two age groups. In previous studies, moduli were found to increase with cartilage maturity in humans up to 30 years of age (6), while decreases in moduli are commonly observed with osteoarthritis in humans and larger animal models (6,7,8,9). With this osmotic loading technique, matrix properties are measured in the thickness direction as compared to tensile studies of cartilage that evaluate moduli in a direction tangential to the articular surface. Thus, our findings for mechanical stiffness may not directly compare with those reported previously. In the 2-month guinea pigs, cartilage may still be maturing since histological and SEM measures of aging have not been observed until 3 to 4 months of age (10). With the most degenerate cartilage, however, the measured properties were more variable in the 12-month group, which may reflect varying degrees of degeneration. Studies are ongoing from 12 to 18 months of age to ascertain material properties within a timeframe when osteoarthritic changes predominate.

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