INTRODUCTION
Dunkin-Hartley guinea pigs undergo spontaneous cartilage degeneration, and they have been used as a convenient small animal model to study osteoarthritis (OA). Detailed stereological analysis of histologic sections of guinea pig knee joints have revealed a site-specific increase in cartilage fibrillation between 6 and 60 months of age. The cartilage fibrillation that accompanies OA can be visualized as areas staining with India ink, and the intensity of staining has been related to local changes in the biochemical and biomechanical properties of cartilage. Quantitative imaging of ink-stained human joints has been used to delineate the extent of cartilage degeneration and to identify age-related increases in the knee. Calibrated digital image analysis of ink-stained rabbit joints subjected to experimental ACL transection has been used to determine the area-averaged extent of ink staining and also to localize areas prone to degeneration. The objectives of this study were to determine whether India ink staining and digital imaging methods could be used to identify age- and site-dependent degeneration of cartilage in the knee joints of guinea pigs.

METHODS
Specimens. Nine male Dunkin-Hartley strain guinea pigs were obtained from C.R. Canada at 2-3 months of age. At 7, 11 and 15 months of age, animals were sacrificed with pentobarbital (200 mg/kg IP), and the femora and tibiae were harvested and frozen at -20°C.

India Ink Staining and Image Analysis of Cartilage Degeneration. The specimens were subsequently thawed by immersion in PBS and proteinase inhibitors (PI), mounted in a frame, and positioned to obtain a view of the distal femoral condyle or tibial plateau, with the bone at a 45° angle from the vertical axis. Each specimen was painted with 20 µl of a solution of India ink, diluted 1:5 with PBS+PI, and, after 15 s, wiped with moist tissue to remove excess ink. Using a digital video system, each specimen was imaged and analyzed using NIH Image software. For standardization, a calibration grid with known length scales and grayscale reflectance squares (0=black and 1=light gray #3, Kodak Q13) was imaged with each specimen. Light reflectance from regions encompassing most of the cartilage of the medial and lateral femoral condyle (MFC, LFC; 25.0 mm²) and tibial plateau (MTP, LTP; 26.7 mm²) was measured in each of the individual specimen images and normalized according to the grayscale calibration values. Images from the right knee were flipped digitally in the horizontal plane so that they could be processed with the left knee images. Digital averages of the registered femoral and tibial images from each experimental group were computed (Fig. 1A-F). To highlight and localize differences in ink-staining patterns with aging, difference images were computed (Fig. 2A-D).

Statistical Analysis. Light reflectance data are expressed as mean ± SD. Multivariate ANOVA and Tukey post-hoc test were used to assess the effects (fixed factors) of age (7, 11, and 15 mo.), joint (tibia and femur), and aspect (medial and lateral), as well as the effects (random factor) of differing animals on reflectance scores.

RESULTS
The averaged (Fig. 1) and difference (Fig. 2) images showed areas of cartilage degeneration with increasing age. Ink staining increased from 7 mo. to 11 mo. in the LFC, FTP and MTP, and also from 11 mo. to 15 mo. in the LFC, MFC and MTP.

Quantitative reflectance scores from the central cartilaginous regions of each cartilage surface varied with age (ANOVA p<0.001, Fig. 3). The decreases in reflectance scores between 7-mo. and 11-mo. specimens as well as between 11-mo. and 15-mo. specimens were significant (p<0.01 and p<0.005, respectively). Overall, the TP showed more degeneration than the FC (p<0.001), and the medial side showed more degeneration than the lateral side (p<0.001).

DISCUSSION
This study sensitively mapped and quantified the degenerative changes in the articular cartilage of Dunkin-Hartley guinea pigs. Quantitative image analysis of articular cartilage provided an objective measure of cartilage degradation over large areas of the knee joint. These image analysis results are consistent with previous histological studies that described degenerative changes as occurring in the MTP by 6 mo. of age, and progressing markedly in both the MTP and LTP by 12 mo. of age. The localization of cartilage degeneration may be particularly useful for assessing compositional, structural, or functional cartilage changes at various stages of degeneration. Also, quantitation of the extent of degeneration may be particularly useful for evaluating therapeutic interventions.

REFERENCES

**Genentech, Inc., South San Francisco, CA.