INTRODUCTION
Recent immunohistochemical studies have demonstrated that chondrocytes in human and canine articular cartilage can express the gene for a contractile muscle actin isoform, α-smooth muscle actin (SMA) (1, 2). SMA-expressing human and canine articular chondrocytes were found to contract a collagen-glycosaminoglycan (CG) analog of extracellular matrix. These findings prompted the current study to quantify the force of contraction generated by articular chondrocytes employing a novel cell force monitor (3). Associated studies were undertaken to directly image cell-matrix interactions in the same time frame in order to reveal the process by which the cells cause contraction of the cell-seeded collagen-glycosaminoglycan scaffold, using a method previously employed for fibroblast-seeded scaffolds (4).

MATERIALS AND METHODS
Cell Isolation and Treatment
Articular cartilage was harvested from the knee (stifle) joints of an adult dog and dissected into 0.5-0.1 mm 3 pieces. Cells were isolated and cultured in 10% FBS-supplemented DMEM/F12. In order to modulate the actin cytoskeleton, samples of passage 3 cells were treated with staurosporine (5 nM), an antibiotic known to inhibit protein kinase C and disrupt cytoskeletal structure. SMA Western Blot Analysis
SMA Western blot analysis was performed as previously described in detail (4). 15μg of protein was run vs. 5μg for the smooth muscle cell (aorta) controls.

CG Matrices
The production of type I CG copolymer sponge-like matrices has been previously described in detail (5). Briefly, type I bovine tendon collagen (Integra LifeScience, Plainboro, NJ) was mixed with chondroitin 6-sulfate. The slurry was freeze-dried to produce 3-mm thick sheets that were dehydrothermally cross-linked.

DISCUSSION
Adult canine articular chondrocytes were capable of generating forces of at least 0.3 nN when seeded in CG matrices. This compared to values of approximately 1nN per cell for fibroblasts investigated in the same apparatus (3). Considering that all of the cells in the CG matrix were not contributing to the force that was recorded, the force per chondrocyte was likely substantially higher that that estimated from dividing the total force by the total number of cells in the matrix. Passage 3 chondrocytes generated a significantly higher force than passage 2 chondrocytes due to the increased content of SMA. Stauorosporine, a disruptor of the cytoskeleton, decreased the force generated by passage 3 articular chondrocytes. This reduction in the generated force also coincided with a decrease in SMA expression, consistent with prior work (4). This is the first report of live cell imaging of chondrocytes in a CG matrix. We were able to correlate the forces generated by the cells with the observed cell-matrix interactions. The process of chondrocyte-mediated contraction involved the elongation of the cells along the beams and struts making up the CG scaffold. This was followed by cell contraction and the shortening of the thinner beams, thus drawing together the attached struts, and producing the force being recorded in the cell force monitor. The contractile forces generated by articular chondrocytes may be of importance in enabling their modeling of cartilage matrix synthesis in vivo.

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REFERENCES

Forces Generated by the Articular Chondrocyte in the Collagen-GAG Matrix
The peak force per cell measured for the passage 3 chondrocytes was about 0.3 nN and was reached after about 3 hours. The third passage cells generated about twice the force recorded for the passage 2 cells, also after about 3 hours (Fig. 1). This difference was statistically significant (ANOVA). Treatment of the passage 3 chondrocytes with staurosporine significantly reduced the force generated by the cells to approximately one-half the level (Fig. 1). Live Cell Imaging documented the contractile behavior of the articular chondrocytes in the collagen-glycosaminoglycan matrix in the time frame in which the force was directly measured in the cell force monitor. This imaging demonstrated how the cells acted individually and in unison to buckle the collagen struts along which they spread and thus draw together connected struts making up the matrix.