INTRODUCTION
Prevention of articular cartilage degradation or promotion of its repair remains a challenge in orthopaedic practice. Therapeutic strategies that rely on the delivery of growth factors to enhance repair or inhibit molecules that would prevent degradation from taking place are of interest. Ideally, the therapeutic protein should be produced in situ, so as to obtain a persistent and stable concentration, thus limiting the need for repeated injections and supraphysiological doses. Gene therapy can achieve this outcome, with adeno-associated virus (AAV) showing promise as a gene delivery vehicle for orthopaedic applications [1]. Intra-articular delivery of AAV is a simple method to deliver genetic material. For development of efficient therapies, it will be important to determine the length of transgene expression, the cells within the joint that uptake AAV, and the ability of the inducible AAV vectors to externally regulate transgene expression. This study was designed to test the hypothesis that in vivo transgene expression mediated by a single intra-articular injection of AAV serotype 2 (AAV2): (1) persists through one year after injection, (2) is present in soft tissues and chondrocytes, and (3) can be externally controlled.

METHODS
Animal experiments were performed following Institutional Animal Care and Use Committee approved protocols. Nine male Sprague-Dawley (SD) rats received a single intra-articular injection (50-µL) containing 2.5 x 10^10 vector genomes (vg) of AAV2-CMV-GFP and AAV2-CMV-Luciferase (Luc) into their right and left knees, respectively. Luciferase expression was evaluated over a 1-year period using the IVIS® 200 Imaging System (Caliper Life Sciences). Bioluminescent flux was reported as photons/second/second. After sacrifice, tissues within the joint were analyzed by fluorescence stereomicroscopy (MVX-10, Olympus) to detect GFP+ cells. To study the ability to externally control AAV transgene expression in the rat knee joint, an inducible AAV vector system was used. Five male SD rats received a 50-µL intra-articular injection containing 2.5 x 10^10 vg of AAV2-tetracycline response element (TRE)-Luc and 2.5 x 10^10 vg of AAV2-CMV-transcriptional transactivator (rtTA) into the right knee. The left knee served as a control and received 2.5 x 10^10 vg of AAV2-CMV-Luc and 2.5 x 10^10 vg of AAV2-CMV-rtTA, to have an equal amount of vg in each joint. To induce expression of luciferase, 2 mg/mL doxycycline (Dox) was added to the drinking water, and rats were imaged twice a week as described above. Data are expressed as mean ± standard error of mean. Comparisons between groups were made using one-way ANOVA with Tukey post-hoc analysis. p < .05 were considered significant.

RESULTS
Luciferase expression, visualized by bioluminescence, was detectable in all rats post-injection. Long-term study showed continued and stable luciferase expression throughout 1 year (Figure 1). No luciferase was observed in other areas of the body, including the contralateral joint. Bioluminescent images of the intra-articular space of the AAV2-CMV-Luc injected knee revealed that the luciferase signal was concentrated in two areas; the infrapatellar fat pad and the soft tissues between the femur and tibia (Figure 2A). Stereomicroscopy of tissues harvested from the knees that received AAV2-CMV-GFP showed that GFP+ cells were mainly found in the intra-articular soft tissues surrounding the articular cartilage (data not shown), with rare GFP+ chondrocytes (Figure 2B).

For the second part of the study, which tested the inducible AAV2 vector, luciferase expression was seen for the duration of the experiment in all knee joints injected with AAV2-CMV-Luc (Figure 3 A, B, and C). Intra-articular injection of AAV2-TRE-Luc led to a weak luciferase signal before addition of Dox (Figure 3A and D). This signal increased as early as 3 days after the addition of Dox to the drinking water of the rats, with a statistically significant increase observed after 7 days (Figure 3B and D, *p = .015 compared to 0 days). Removal of Dox from the drinking water led to a decrease in the signal 3 days later, and a statistically significant decrease was noted after 7 days (Figure 3C and D, #p = .023 compared to Dox (+) at 7 days).

CONCLUSION
AAV2 delivered by a single intra-articular injection to the rat knee joint led to persistent and stable transgene expression that was mainly localized to the soft tissues of the joint, with some expression in chondrocytes. Intra-articular injection of an inducible AAV2 vector demonstrated the ability to regulate in vivo transgene expression by oral administration of doxycycline, a compound that is safely used in humans. These data strongly support potential use of AAV for sustained, stable, and regulated intra-articular release of bioactive factors from soft tissues. Regulating in vivo intra-articular gene expression through orally administered agents will be clinically valuable to control the length of time a therapeutic protein will be expressed and facilitates multiple rounds of administration, without the need for repeated injections. The information obtained in this study provides important insights into the development of safe and controllable AAV-mediated therapies to repair articular cartilage damage or prevent its degradation.