INTRODUCTION: Osteoarthritis (OA) is the most common form of arthritis and one of the leading causes of disabilities, affecting more than 20 million people in the US. Several biomolecules are depleted in an OA joint including hyaluronic acid (HA) in the synovial fluid and aggrecan in articular cartilage. Current conservative treatments for OA aim to slow the progression of the disease and mediate pain with analgesics, anti-inflammatory drugs, physical therapy and weight loss. Intra-articular HA injections (Synvisc®, Orthovisc®, Euflexxa®) have been widely used in an attempt to restore joint lubrication and reduce cartilage erosion, however their effects have resulted in limited success. The American Academy of Orthopaedic Surgeons recently (2013) withdrew support for HA injections due to the limited efficacy. HA injections do not repair the cartilage itself. Our lab has synthesized a novel biomimetic aggrecan (BA) aimed to infiltrate and distribute in articular cartilage through a minimally invasive injection to the synovial joint space. BA is intended to restore the hydration and mechanical function to arthritic cartilage by molecularly engineering the tissue without relying on cellular synthesis to regenerate the extracellular matrix. BA10 mimics the 3-D bottle brush structure and properties of naturally occurring aggrecan and consists of a poly(acrylic acid) (10kDa) core with chondroitin sulfate bristles. Using BA10, we propose a long-lasting, minimally invasive treatment for OA that will provide increased mobility and pain relief to the joint and molecularly repair the damaged cartilage.

METHODS: Six female New Zealand White Rabbits age 20-24 weeks were used in this IACUC approved pilot study (Protocol #20349). ACL Transection (ACLT) was used to create osteoarthritis in the right hindlimb of 4 rabbits while the left hindlimb of each rabbit was used as a normal control. The rabbits were divided into 2 groups: 1) ACLT alone and 2) ACLT + BA10. Five weeks after the surgery, the two rabbits in the ACLT + BA10 group underwent a series of weekly injections for 3 weeks. BA10 (180kDa) was suspended in a 1XPBS solution, sterilized and prepared for injection. Two non-ACLT additional rabbits underwent BA10 injections in the right hindlimb, with injections being performed once weekly over a period of 3 weeks. For the same rabbits, the contralateral limb had one injection of fluorescently-labeled BA10 two days prior to sacrifice to visualize labeled BA infiltration into the cartilage extracellular matrix. The hindlimbs were removed immediately after euthanasia for mechanical testing and then fixed for histology. The muscle and soft tissue were removed from the limbs and they were mounted individually to a custom built pendulum friction tester with the knee joint serving as the fulcrum of the pendulum. The pendulum was allowed to oscillate freely and the motion was analyzed with Matlab to obtain the coefficient of friction of the knee joint. The hindlimbs were then further dissected, fixed, decalcified, sectioned, and stained with safranin-O. The resulting stained slides were viewed under an AMG EVOS microscope and scored using the Mankin score. Unstained slides from the fluorescently labeled BA10 injected limbs were viewed with a DAPI filter on an Olympus FV1000 confocal microscope to detect where the BA10 molecules were within the articular cartilage.

RESULTS: Osteoarthritic rabbit knees treated with BA10 exhibited a lower coefficient of friction compared to OA knees alone. The rabbit knees which only received BA10 injections exhibited almost the same coefficient as the control knees. A more robust articular cartilage layer is present in arthritic specimens treated with BA than the arthritic specimens with no treatment. Confocal microscopy of the fluorescently labeled BA10 injected knees showed fluorescence within the articular cartilage indicating diffusion of BA10 into the cartilage matrix. Our pilot results indicate that BA10 decreases the coefficient of friction of the osteoarthritic knee and increases the cellularity and thickness of the articular cartilage, supporting our hypothesis that BA can provide increased hydration to the joint and regenerate superficial layers of articular cartilage. The results from our pilot study provide preliminary data to support the ability of biomimetic aggrecan to molecularly engineer osteoarthritic cartilage through infiltration of BA from the synovial joint into the articular cartilage layer. BA has implications not only for mechanical restoration of the joint, but also for localized drug delivery to cartilage. Our preliminary findings on joint friction indicate that BA has no adverse effect on joint coefficient of friction and may help to reverse the increased friction that resulted from OA. A study using the same model is currently underway with a group size of five.

SIGNIFICANCE: BA may have efficacy as a minimally invasive treatment option for patients with early stage osteoarthritis by its ability to provide increased hydration to the joint, reduce friction of an osteoarthritic knee, and to regenerate superficial layers of articular cartilage.


ACKNOWLEDGEMENTS: We would like to thank the Clinical and Translational Research Institute at Drexel for funding this project and Richard B. Huneke, DVM, LATG, and Emily Reimold, MLAS, LATG, for their assistance with the project.

Figure 1: A-D show safranin-O stained slides for A) control, B) OA, C) BA10 only, and D) OA+BA10. E and F show the fluorescent (left) and contrast (right) image for E) fluorescently labeled BA10 and F) control. The fluorescent image for E was enlarged to remove the fluorescence from the folding artifact. The graph on the right shows the coefficient of friction values for the 4 groups tested.