# Anakinra Protects Against Mechanical Degradation of Articular Cartilage in an *In Vitro* Inflammatory Environment

Emily S. Cook[1,2], Brendan D. Stoeckl[1,2], Austin C. Jenk[1,2], David R. Steinberg[1,2], Robert L. Mauck[1,2]
[1]University of Pennsylvania, Philadelphia, PA. [2]CMC Veterans Administration Medical Center, Philadelphia, PA.
esc6@seas.upenn.edu

**Disclosures:** RLM (5- 4WEB Medical)

**INTRODUCTION**: Articular cartilage is essential for the proper mechanical function of the knee, but is prone to degradation following injury and exhibits poor healing capabilities. These injuries can lead to a "red knee" inflammatory environment [1], which exacerbates damage and hinders clinical cartilage repair strategies. Recently Anakinra, a recombinant version of Interleukin-1 receptor antagonist (IL-1Ra) has shown promise as a therapeutic to reduce post-traumatic arthritis [2-4] and limit disease progression. We recently showed that application of Anakinra in a large animal model of osteochondral repair (using the osteochondral autograft transfer system (OATS) procedure, in which cartilage is explanted from a non-bearing region and implanted into the damaged load-bearing region), improved graft functional properties five weeks following implantation [5,6]. However, given the complicated inflammatory and mechanical post-surgical environment, it was difficult to say with certainty whether the specific effects of Anakinra were on the articular cartilage or working through other joint structures (i.e., synovial inflammation, boney remodeling). To directly test the effects of Anakinra on cartilage functional properties, here we cultured cartilage *in vitro* in media containing either IL-1β alone or a combination of IL-1β and Anakinra. We hypothesized that provision of Anakinra would preserve the mechanical properties of cartilage cultured in a simulated inflammatory environment.

**METHODS**: Articular cartilage explants were sterilely isolated from juvenile bovine femoral condyles using a 4mm diameter biopsy punch and cut into cylinders 2mm in height from the cartilage surface. Explants were pre-cultured in basal media (BM) for 4 days before media was supplemented at day 0. Explants were split into 4 groups, with explants from each joint (N = 6 joints, ~16 explants/joint) split evenly across all 4 groups (N = 8-10/group/timepoint): Control (BM only), +IL-1β/-Anakinra (BM + 50ng/mL IL-1β), -IL-1β/+Anakinra (BM + 1000ng/mL Anakinra), and +IL-1β/+Anakinra (BM + 50ng/mL IL-1β + 1000ng/mL Anakinra). Media was changed and supplemented 3 times/week and explants were removed from culture on day 0 (baseline Control) and days 7 and 14 (all groups). Explants were frozen in 1x PBS at -20˚C until mechanical testing, at which time they were thawed and immersed in PBS in a custom fixture on an Instron mechanical test frame. Creep indentation testing [7] was performed on all samples at a constant force of 0.1N for 15 minutes, after which samples were allowed to swell to their original thickness prior to refreezing at -20˚C. After testing, samples were then thawed and split into sections for biochemical assays and histology. Samples for biochemical analysis were digested overnight with Proteinase-K followed by measurement of GAG content via a DMMB assay and DNA content via a PicoGreen assay [8]. Samples for histology were fixed in 10% formalin, processed into paraffin, and sectioned to 10 microns. Staining included Safranin O/Fast Green and Alcian Blue/Picrosirius Red [9]. All quantitative data was analyzed in GraphPad Prism using a Kruskal-Wallis test ($p<0.05$) followed by Dunn's multiple comparison post hoc test between groups at each timepoint.

**RESULTS**: Treatment with IL-1β significantly altered cartilage explant mechanical properties over time, including a reduction in compressive and tensile moduli and an increase in permeability by day 14. While treatment with Anakinra protected against IL-1β mediated-degradation, the compressive modulus and permeability were more noticeably affected than the tensile modulus (**Fig. 1**). Notably, application of Anakinra alone (in the absence of IL-1β) did not protect against the progressive loss of properties that explants experience during free-swelling culture over 2 weeks. That is, properties in the IL-1β/+Anakinra group on day 14 were significantly different from controls at day 0 ($p=0.001$). Histological analysis showed that IL-1β treatment significantly degraded the cartilage throughout the full thickness of the cartilage explants (**Fig. 2A, B**) and reduced the concentration of glycosaminoglycans (GAGs), as seen in both Alcian Blue staining (**Fig. 2C**) and direct quantification of GAG content (**Fig. 2D**). Samples treated with +IL-1β/+Anakinra retained more proteoglycans/GAG than the +IL-1β/-Anakinra treated samples ($p<0.0001$ for staining quantification and $p=0.0033$ for GAG content) and did not differ significantly from the control (-IL-1β/-Anakinra) group in either measure.

**DISCUSSION**: Here, we demonstrate the protective effects of Anakinra, an IL-1 receptor antagonist, against inflammation-induced degradation of cartilage and loss of mechanical integrity. Although explant cartilage samples experienced some loss of mechanical properties in culture over the 14-day period as expected [10], supplementation with Anakinra was sufficient to protect the cartilage from significant degradation across all outcomes, including mechanical properties and GAG content, when exposed to IL-1β. While this study focused primarily on the macroscale effect of Anakinra in an inflammatory environment, it will be important to investigate microscale changes in extracellular matrix mechanics to determine whether treatment preserves both depth-dependent and pericellular mechanics of the native tissue. Additionally, while the work presented here shows that Anakinra protects against IL-1β-induced degradation when administered at the same time, future studies will investigate whether Anakinra can rescue (or stabilize) mechanical properties when administered hours or days after inflammation begins, to more accurately represent a realistic injury state. Importantly, however, this study does support our initial hypothesis that Anakinra can protect articular cartilage from the loss of mechanical integrity and consequently supports the use of Anakinra as an adjuvant therapeutic during *in vivo* cartilage repair procedures.

**SIGNIFICANCE**: This *in vitro* investigation of the protective effects of Anakinra against cartilage degradation in an inflammatory environment supports further *in vivo* studies of its application as an adjuvant therapeutic used in combination with cartilage repair procedures to improve outcomes in the "red knee" joint environment.

**REFERENCES**: [1] Martín+ *NPJ Regen Med*, 2019. [2] Chevalier+ *Arthritis Care Res*, 2009. [3] Kraus+ *Osteoarthr Cartil*, 2012. [4] *Caron+ Arthritis Rheum*, 1996. [5] Stoeckl+ *ORS,* 2025. [6] Stoeckl+ *SB3C,* 2025. [7] Moore+ *J Tribol*, 2016. [8] Huang+ *Eur Cell Mater*, 2012. [9] Stoeckl+ *Acta Biomater*, 2021. [10] Meloni+ *Eur Cell Mater*, 2019.

**ACKNOWLEDGEMENTS**: This work was supported by the NIH (R01 AR077362 and P30 AR069619) and the VA (I50 RX004845, I01 RX003375, and I21 RX004628).

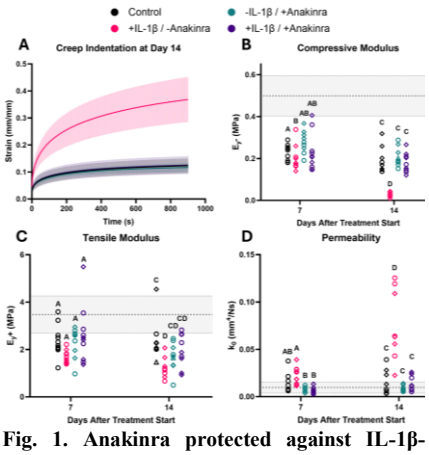


**Fig. 1. Anakinra protected against IL-1β-mediated loss of mechanical properties.** (A) IL-1β reduced resistance to creep deformation compared to all other groups. IL-1β lowered the compressive (B) and tensile moduli (C) and increased permeability (D) of the cartilage, while co-supplementation with Anakinra somewhat (C) or significantly (B, D) protected the cartilage mechanical properties. Letters indicate significant differences between groups; $p<0.05$. Grey box indicates day 0 control values; mean +/- SD.

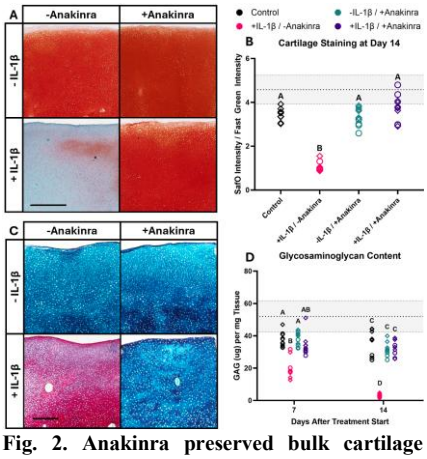


**Fig. 2. Anakinra preserved bulk cartilage matrix proteoglycans**. Safranin-O (A, B) and Alcian Blue staining intensity (C) was reduced by IL-1β treatment but preserved by treatment with both IL-1β and Anakinra. GAG content (D) was similarly affected by IL-1β, while Anakinra co-supplementation protected against GAG loss. Letters indicate significant differences between groups; $p<0.05$. Scale = 500 microns. Grey box indicates day 0 control values; mean +/- SD.