

# $\alpha_2$ -Macroglobulin Reduces ACL Degeneration in a Posttraumatic Osteoarthritis Environment in the Yucatan Minipig

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**INTRODUCTION:** Posttraumatic osteoarthritis (PTOA) is characterized by cartilage degeneration following joint trauma, such as intra-articular fractures, meniscus tears, and anterior cruciate ligament (ACL) ruptures. PTOA pathogenesis is attributed to a combination of mechanical alterations and chronic inflammatory catabolism. Although the effects of pathologic inflammatory mediators have been well studied on cartilage, the impacts on other intra-articular structures, such as the ACL, have not. Recently, our group developed the modified intra-articular drilling (mIAD) model in the Yucatan minipig, which induces cartilage degeneration through inflammatory pathways without damaging intra-articular soft tissues or causing detectable changes in joint biomechanics.<sup>1</sup> We noticed that this inflammatory PTOA environment also induced ACL degeneration. This model allows for the specific investigation of inflammation and inflammation-targeted therapies. In prior animal studies, we found that alpha-2-macroglobulin ( $\alpha_2$ M) effectively reduces cartilage degeneration by reducing inflammation. Thus, to further understand the impacts of inflammation and potential therapies for soft tissue structures, the objective of this study was to investigate the effects of intra-articular injections of  $\alpha_2$ M on ACL degeneration in Yucatan minipigs that had undergone mIAD. We hypothesized that minipigs treated with  $\alpha_2$ M would have less ACL degeneration and cell density than untreated minipigs but similar outcomes as the sham arthrotomy group.

**METHODS:** The study was approved by the IACUC. For this study, cartilage and ACL specimens from Yucatan minipigs were obtained from a subset of a previous porcine study of the mIAD model. 23 Yucatan minipigs (age 15-16 months old, weight 54.7±6.2kg) were split into four groups: (1) mIAD with three control saline intra-articular injections (mIAD+saline, n=7), (2) mIAD with one  $\alpha_2$ M and two saline injections (mIAD+ $\alpha_2$ M-1, n=6), (3) mIAD with three  $\alpha_2$ M injections (mIAD+ $\alpha_2$ M-3, n=6), and (4) sham arthrotomy with three saline injections (n=4). Animals were anesthetized, and a medial arthrotomy was performed on the left hind knee to gain access to the ACL. For animals undergoing mIAD, two osseous tunnels 2mm wide and 15 mm deep were drilled into the tibial bone adjacent to the anterior and posterior insertions of the ACL. The drilling procedure was repeated at the anterior medial and posterior lateral edges of the femoral ACL insertion. The sham group underwent the same procedure but without the drilling. Intra-articular injections were performed immediately after, 2 weeks after, and 4 weeks after surgery. Load asymmetry during gait was recorded by a pressure-sensing walkway measurement system (Tekscan) before (0 weeks) and after surgery (4, 8, 12, and 15 weeks). The differential weight bearing between the left (surgical) versus right (control) hind limb was expressed as a ratio. Surgical hind knees and ACLs were harvested 15 weeks post-surgery. Medial and lateral sites of the femoral condyle and tibial plateau were evaluated histologically using the OARSI scoring system. ACL degeneration was evaluated using a histological scoring system described by Kharaz et al.<sup>2</sup> Data were compared using the Kruskal-Wallis test. Post-hoc comparisons were performed with the Benjamini-Hochberg adjustment.

**RESULTS:** The mIAD+saline group had significantly higher OARSI scores, which are a measure of severity of cartilage degeneration, than the mIAD+ $\alpha_2$ M-1 (p=.013), mIAD+ $\alpha_2$ M-3 (p=.031), and sham (p=.048) groups (Fig. 1). Treatment with  $\alpha_2$ M significantly reduced ACL degeneration and cell density in the mIAD+ $\alpha_2$ M-1 and mIAD+ $\alpha_2$ M-3 groups compared with mIAD+saline (Fig 2). In addition, there were no significant differences in all outcomes between the mIAD+ $\alpha_2$ M-1, mIAD+ $\alpha_2$ M-3, and sham groups. In general, there were no significant differences in limb asymmetry from pre-operative to post-operative time points among the 4 groups (Fig 3).

**DISCUSSION:** The results support the hypothesis that intra-articular injections of  $\alpha_2$ M reduce ACL degeneration in a PTOA model driven by inflammation to a point similar to the sham arthrotomy group. Conversely, untreated animals undergoing mIAD had significantly greater cartilage and ACL degeneration. The significantly greater microscopic damage of the ACL in the mIAD+saline group emphasizes that the neighboring soft tissues likely undergo degenerative processes similar to that of cartilage due to inflammation, which may precede detectable changes in biomechanical joint loading. Moreover, the anti-inflammatory properties of  $\alpha_2$ M, likely through its inhibition of cytokines and proteases, can effectively attenuate ACL degeneration.

**SIGNIFICANCE/CLINICAL RELEVANCE:** This study suggests that  $\alpha_2$ M may effectively attenuate soft tissue degeneration, specifically the ACL, by inhibiting the catabolic cascades involved in cartilage degeneration in an injured joint. The multi-faceted therapeutic potential of  $\alpha_2$ M on soft tissue health should be evaluated clinically in injuries such as intra-articular fractures.

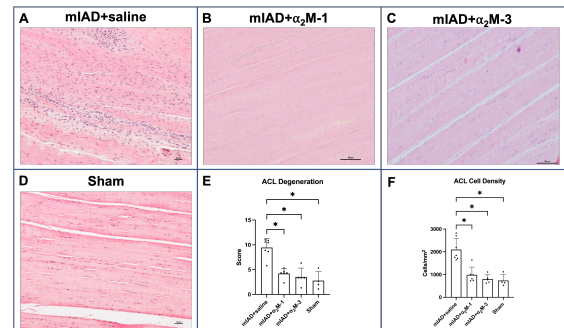
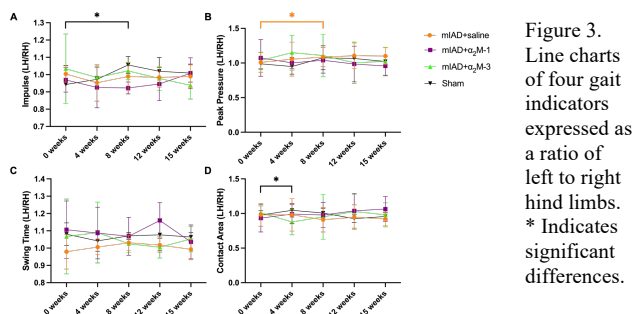
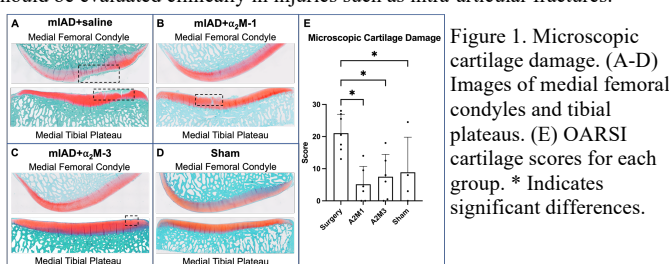


Figure 2. ACL Degeneration. (A-D) Images of the ACL for each group. (E) ACL integrity scores. (F) Cell density of the ACL. \* Indicates significant differences.

## References

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