Orthopaedic Surgery
School of Medicine
University of Missouri Health

Thompson Laboratory for Regenerative Orthopaedics

# Investigation of the Effect of IL-1β, RANTES, and MMP-1 Injection into Nucleus Pulposus of Intervertebral Disc

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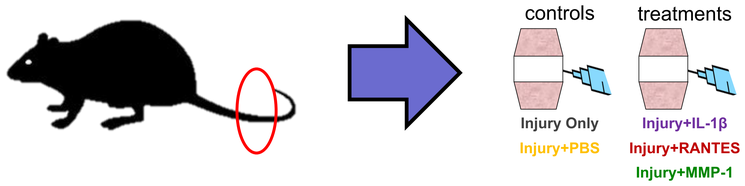
## Introduction and Purpose

- Inflammatory stimulation and degradative enzyme activity contribute to development and progression of symptomatic intervertebral disc degeneration (IVDD)
- IVDD often develops through changes in nucleus pulposus (NP) structure compromising the function of the intervertebral disc (IVD)
- The metabolic effects of injury and localized inflammation and degradative enzyme activity on the NP is poorly understood
- This study was designed to determine the effects of NP stimulation with inflammatory cytokines (IL-1β and RANTES) and a degradative enzyme (MMP-1)

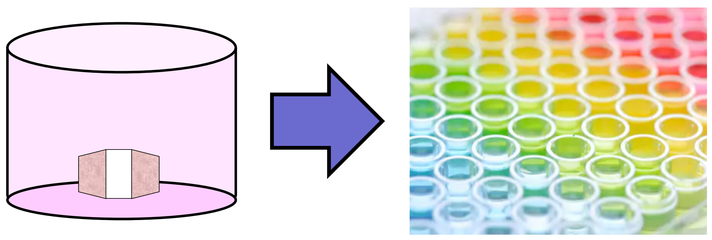
## Hypotheses

- Localized stimulation of the NP in rat tail IVDs will significantly increase production of pro-inflammatory and degradative biomarkers
- Injection of inflammatory and degradative agents will significantly decrease biomechanical properties of rat tail IVDs

## Methods




1) With ACUC approval, rat tail IVD explants were created, and randomly assigned either control (injury only or PBS [Injury + Injection]) or treatment (IL-1β, RANTES, or MMP-1 [Injury + Injection]) groups
2) A 25G needle was used to create an injury and inject 10μl of solution based on group.

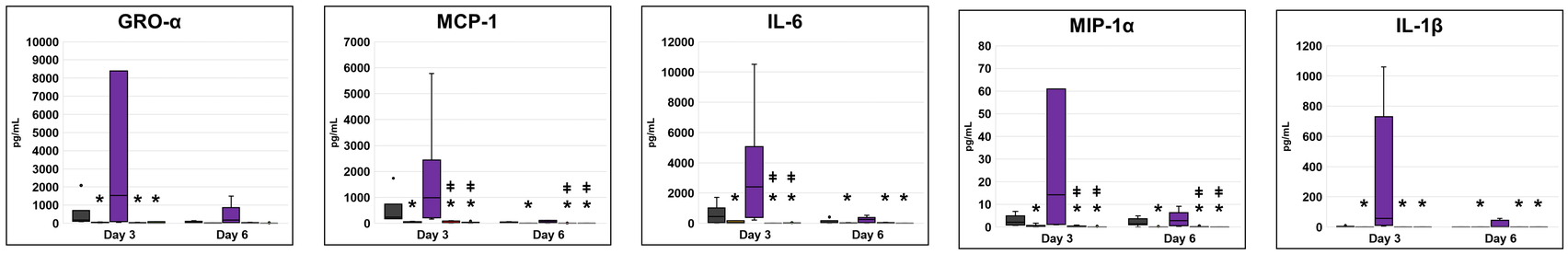


3) Media from days 3 and 6 were collected and measured for MIP-1α, IL-6, IL-13, IL-10, MCP-1, GRO, VEGF, IFN-γ, IL-1β, IL-4, TIMP-1, TNF-α, RANTES, IGF-1, MMP-8, FGF-β, and MMP Activity.
4) Tissue biomechanics were analyzed, and stress, strain, and creep modulus were determined.
5) Histological analysis was done to determine the tissue's ECM properties. Any irregularities in tissue structure were documented.
6) Significant differences between specific groups were assessed at each time point using an ANOVA and Tukey Post Hoc analysis with significance set at $p < 0.05$.

## Results

### Media Biomarker Concentrations

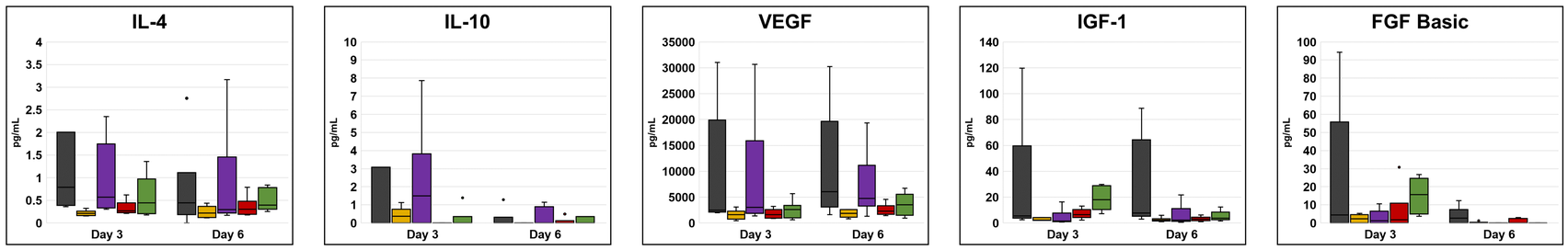
Injury Only | Injury+PBS | Injury+IL-1β | Injury+RANTES | Injury+MMP-1




***Pro-Inflammatory Biomarkers***

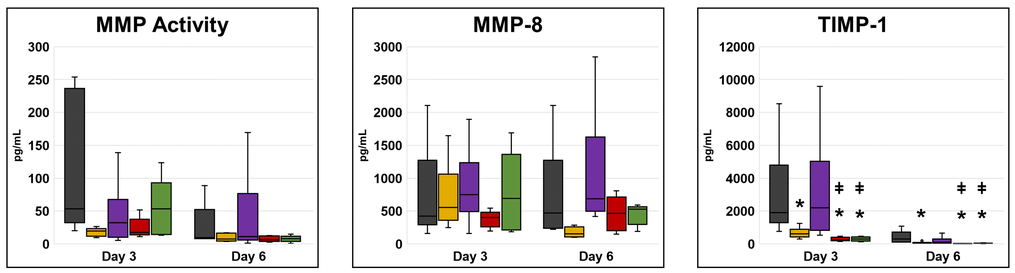
- The Injury+IL-1β group produced significantly higher levels of MIP-1α, IL-6, MCP-1, GRO-α, and IL-1β on day 3, and significantly higher levels of MIP-1α, IL-6, MCP-1, and IL-1β on day 6, compared to the other injection groups.
- The injury only group produced significantly higher levels of MIP-1α, IL-6, and MCP-1 on day 3, and significantly higher levels of MIP-1α and MCP-1 on day 6, compared to the Injury+RANTES and Injury+MMP-1 groups.

(*) Significantly lower than Injury+IL-1β group (‡) Significantly lower than Injury only control




***Anti-Inflammatory Biomarkers and Growth Factors***

- There were no significant differences between groups observed for the production of anti-inflammatory biomarkers and growth factors by the IVD in this study at any time point tested

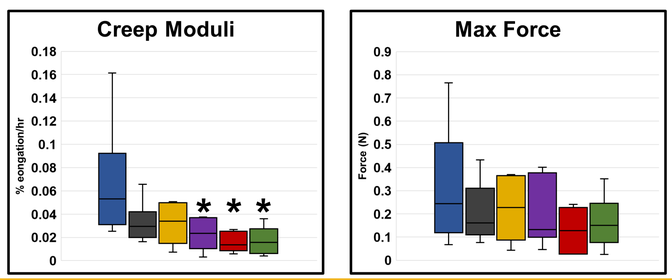



***Degradation-Related Biomarkers***

- The Injury+IL-1β group produced significantly higher levels of TIMP-1 on days 3 and 6 compared to the other injection groups.
- The injury only group produced significantly higher levels of TIMP-1 on days 3 and 6 compared to the Injury+RANTES and Injury+MMP-1 groups.

(*) Significantly lower than Injury+IL-1β group
(‡) Significantly lower than Injury only control

### Biomechanical Properties




- The creep moduli in the IL-1β, RANTES, and MMP-1 injection groups were significantly lower than uninjured controls on day 6 of culture
- There were no significant differences in maximum force observed between groups

(*) Significantly lower than uninjured control

## Conclusions

- Localized IL-1β stimulation in the NP, but not RANTES or MMP-1, increased inflammatory response by the IVD
- Injection of PBS in the NP after injury may decrease the pro-inflammatory responses of the IVD after injury
- Localized IL-1β, RANTES, and MMP-1 stimulation in the NP decreased the biomechanical properties of the IVD

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