Effect of Static Load on Intervertebral Disc Viability, Tissue Composition, and Metabolism

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Introduction

Lower back pain related to intervertebral discs (IVDs) is more prevalent than ever and currently places an economic burden on the healthcare system in excess of $50-100 billion dollars, annually. Currently, there are no effective treatments for restoration of IVD function or tissue integrity. In order to better understand the process of disc degeneration and to seek methods of prevention or reversal of damage, in vitro models of disc degeneration have been created. For this study, a custom designed bioreactor was created to apply a static load to the IVD to create a degenerate IVD in vitro model. It is theorized that the response of the IVD to this static load will give insight into the pathogenesis of IVD degeneration in vivo. This model can then be used to design and evaluate new potential treatments for IVD degeneration.

Objective
- **To create an in vitro IVD degeneration model**

Hypothesis
- **A static 1 MPa load applied to the IVD for a duration of 3 days will decrease cell viability, and increase the production of inflammatory and degradative biomarkers, compared to controls.**

Materials & Methods

All procedures were performed under IACUC approval. Lumbar spines were harvested from 8 dogs euthanized for reasons unrelated to this study. Soft tissue was dissected aseptically from the spinal segments, and IVDs were harvested using a diamond band saw. Whole organ explants were harvested (Figure 1) and cultured in a custom designed bioreactor (Figure 2) for 3 days in 10 mL of supplemented culture media. IVDs were randomly assigned one of five Groups: (1) Control (0 MPa), (2) <0.1 MPa (3) 0.1 MPa or (4) 1 MPa. After 3 days of culture, media was collected for biomarker analysis, and IVDs were collected for cell viability analysis. The IVDs bisected, stained using a fluorescent cell viability assay (Invitrogen), and images were taken using a fluorescent microscope. The viability of the nucleus pulposus was assessed subjectively. The viability of the annulus was assessed objectively using an in house developed program to count live and dead stained cells. Data is reported as total number of cells counted per section. Media was analyzed using commercially available assays for IL-6, IL-8, KC, MCP-1, MMP-1, MMP-2, MMP-13, and PGE_2. Data was analyzed for statistical significance using a T-Test with significance set at $p<0.05$.

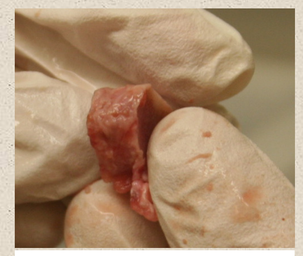


Figure 1: IVD Explant

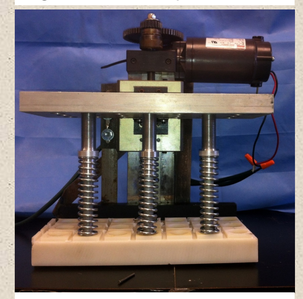
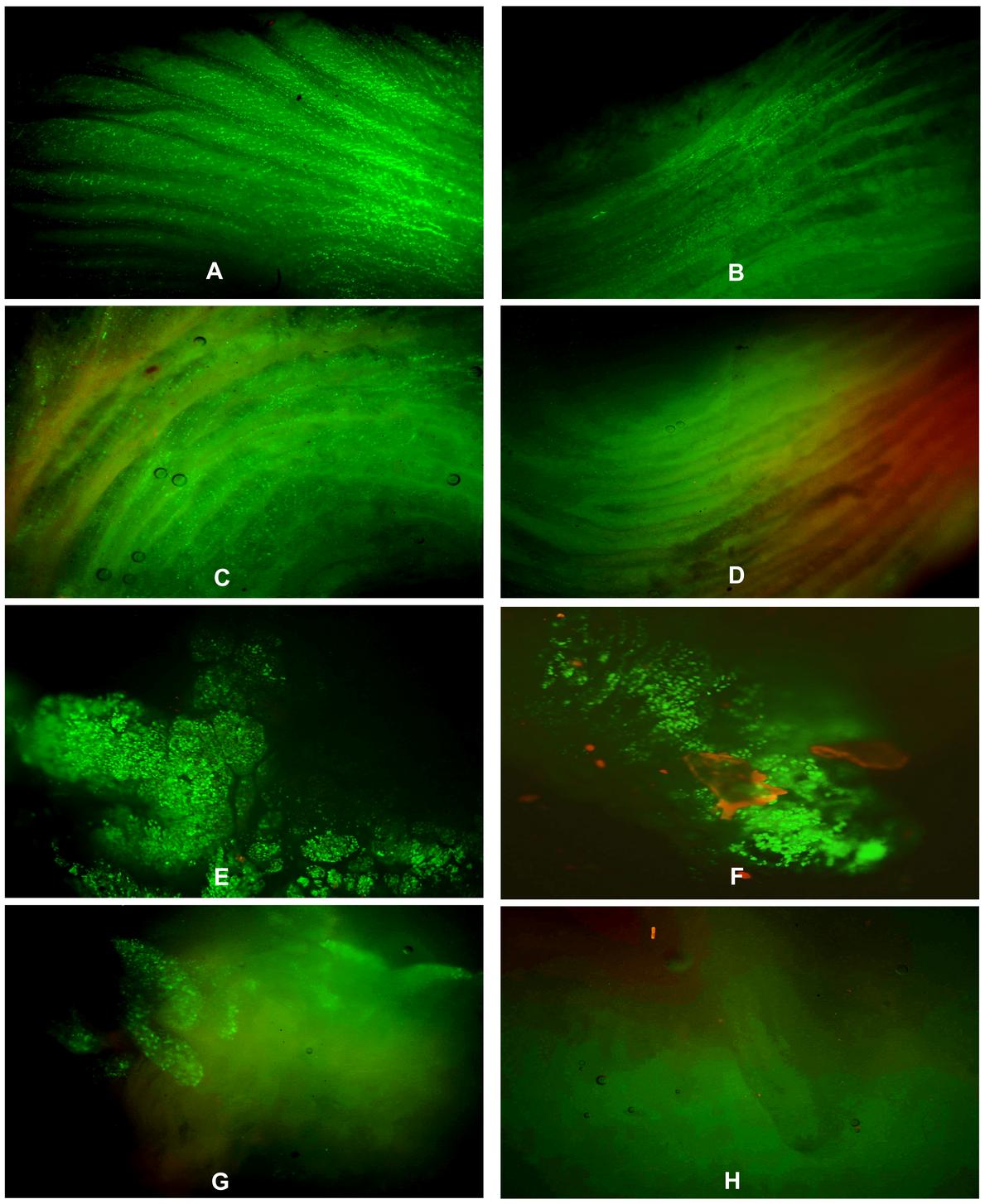


Figure 2: Custom Bioreactor

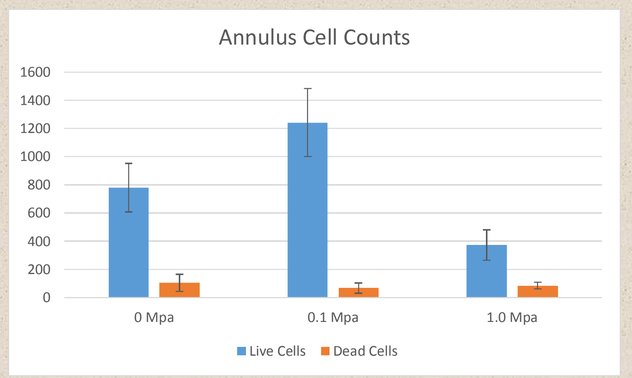
Results

Representative Cell Viability Images

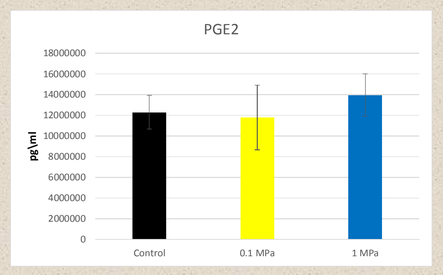


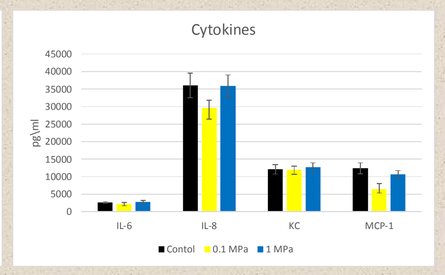
Annulus Fibrosus Viability
- A= Control (Day 0)
- B= 0 MPa (Day 3)
- C= 0.1 MPa (Day 3)
- D= 1 MPa (Day 3)

Nucleus Pulposus Viability
- E= Control (Day 0)
- F= 0 MPa (Day 3)
- G= 0.1 MPa (Day 3)
- H= 1 MPa (Day 3)

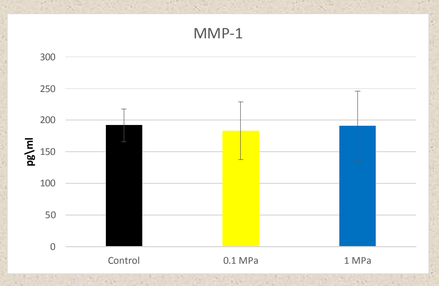


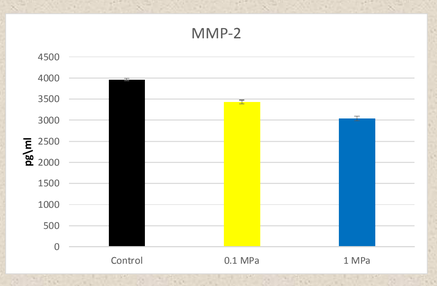
- There was significantly lower viability in the 0 MPa, and 1.0 MPa as compared to 0.1 MPa group

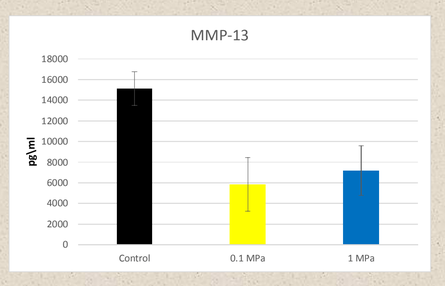




- There were no significant differences between groups for PGE_2, IL-6, IL-8 or KC
- There was a significantly lower production of MCP-1 in media for the 0.1 MPa group as compared to the Control and 1.0 MPa group







- There were no significant differences between MMP-1 or MMP-2
- There was significantly lower production of MMP-13 for the 1 MPa group as compared to the control group
- There was a lower production of MMP-13 between control group and 0.1 MPa group that approached statistical significance $p=0.059$

Discussion

To our knowledge, this is the first study to investigate the effects of a static load upon a whole organ IVD for a period of 3 days. The application of a 1 MPa static load to the IVD resulted in a significant drop in the viability of the tissue compared to the 0 MPa Control and the 0.1 MPa group. A similar loss of cell vitality was not observed in the 0.1 MPa. This indicates that the application of 1.0 MPa load could be a potential model for IVD degeneration. While detectable levels of the biomarkers analyzed could be detected in the media, there were no significant changes in the biomarker levels analyzed resulting from the application of the static load. Therefore further study is required to determine the best methodology for assessing the metabolism of the tissue in this whole organ in vitro model. Future studies will be focus on continued development of this in vitro model and the identification of appropriate biomarkers and testing strategies for the assessment of disc degeneration.

Conclusions

- Application of 1 MPa static load causes significant cell death, and may be an appropriate in vitro model for IVD degeneration

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