Effects of Static Loading on Intervertebral Disc using a Whole Organ In vitro Culture Model

James T. Stannard, BS, Aaron Stoker, MS, PhD, Alexis Zallas, BS, Ferris Pfeiffer, PhD, Theodore Choma, MD, MSC, James Cook, DVM, PhD.
University of Missouri Columbia, Columbia, MO, USA.

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Introduction: Intervertebral disc (IVD) disorders are extremely prevalent, and often result in significant pain and disability. Unfortunately, current treatment options are not capable of restoring tissue integrity or function. The exact mechanisms of IVD degeneration are currently unknown, however aging, injury, nutrition, metabolism and mechanical stress are suspected to contribute to disease pathogenesis and progression. Occupational stress has been correlated to the development of IVD degeneration and lower back pain. These stresses include, prolonged standing, walking, and whole body vibration. It has been theorized that extended loading of intervertebral discs may have a direct effect on IVD cells that leads to a loss of viability and tissue structure. The purpose of this study was to investigate the effects of an extended static load upon a whole organ IVD in culture. We hypothesized that extended load would result in a marked loss of cell viability in both the annulus fibrosus and nucleus pulposus of IVD explants compared to controls.

Methods:
Under ACUC approval, lumbar spine segments were collected from 8 dogs euthanized for reasons unrelated to this research. Soft tissue was removed aseptically and whole organ IVD segments consisting of cranial body half, endplate, IVD endplate and caudal body half, were created using a diamond band saw. The bone segments were aspirated using a 30 mL syringe and a 26 gauge needle. Segments were divided into one of 3 culture groups exposed to 1 MPa, 0.1 MPa or no load. The IVDs were cultured for 3 days in supplemented DMEM, and the static load was applied using a custom bioreactor (Figure 1). After 3 days of culture, the IVDs analyzed for cell viability using calcien am (green live stain) and ethidium homodimer (red dead stain) using fluorescent microscopy. The viability of the annulus fibrosus was determined by dividing the number of green staining live cells by the area of the tissue to determine the viable cell density of the tissue (live cells/tissue area μM²). The viability of the nucleus pulposus was assess subjectively. Media was analyzed for IL-6, IL-8, KC, MCP-1, PGE2, MMP-1,-2 and -13 using commercially available assays. Statistical significance was determined using students T-test with significance set at p<0.05.

Results:
All day 0 explants had high cell viability in both annulus fibrosus and nucleus pulposus (figure 2). Subjectively, the 1 MPa static load group had markedly lower cell viability in both the nucleus pulposus compared to the 0 MPa and 0.1 MPa (figure 2). The viable cell density of the annulus fibrosus in the 1 MPa static load group was a significantly than the 0.1MPa (p=0.009) and 0 MPa (p=0.004) after 3 days of culture. Further, the viable cell density of the 0.1 MPa static load group was significantly lower than the 0 MPa group. The concentration of MCP-1 in the 0.1 MPa group was significantly lower than the 1 MPa
(p<0.05) and 0 MPa (p=0.0226) groups after 3 days of culture. No other significant differences in media biomarker concentrations were observed.

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 viable cell density of the tissue compared to the 0 MPa control and the 0.1 MPa group. A similar loss of viable cell density was also observed between the 0.1 MPa group and the 0 MPa group. This indicates that the application of a static load results in a negative effect on IVD degeneration in-vitro. While detectable, the levels of biomarkers analyzed failed to reach significant levels for further data analysis. Therefore, further study is required to determine the best methodology for assessing tissue metabolism of a whole organ IVD. Future studies will focus on the continued development of this in-vitro model and the identification of appropriate biomarkers and testing strategies for the assessment of disc degeneration.

Significance: The data from this study confirms that long-term static loading of the IVD causes significant cell death and is a potential contributing factor in IVD degeneration.
Figure 1: Custom Bioreactor
Figure 2:
- Viable Cell Density

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