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

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

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Introduction:
Intervertebral disc (IVD) disorders resulting in pain and disability are extremely prevalent and current treatment options do not result in restoration of tissue integrity or function. The exact mechanisms of IVD degeneration are not fully understood at this time although aging, injury, nutrition, metabolism and mechanical stress are suspected to contribute. In order to more fully understand IVD degeneration, an in-vitro whole organ model of IVD is desirable. Furthermore, while whole organ IVD models utilizing rats and other small species are prevalent in the literature, canine whole organ IVD models are lacking. It has been theorized that preservation of canine IVDs in-vitro is difficult due to the lack of diffusion of vital nutrients across the endplate. In order to assess the effect of nutrient diffusion, we hypothesized that a diurnal load would be associated with higher cell viability, increased tissue structure and decreased stiffness compared to a static load. We further hypothesized that a load of 1 MPa would be associated with a marked loss of cell viability and tissue structure of IVD explants compared to 0.1 MPa load over a 3 day period.

Methods:
Under ACUC approval, lumbar spine segments containing disc segments L1-L5 were harvested from canine (n=7) after they were euthanized for reasons unrelated to this study. Soft tissue was aseptically dissected from the lumbar spine segments. Explants of cranial body half, endplate, IVD, endplate, and caudal body half were harvested using a diamond band saw. Explants were aspirated using NaCl and then the IVDs were randomly assigned to either a diurnal or static load. In both categories, explants were further divided into either a 0.1 MPa load or a 1 MPa load delivered by a custom built loading apparatus. Explants in the diurnal load group were then placed underneath a load for 12 hours and relaxed for the next 12 hours. Explants in the static load were loaded for the duration of the study. Explants were immersed in Dulbecco’s modified Eagles medium supplemented with essential nutrients. After 3 days, IVDs were bisected and tissue was subjectively assessed using fluorescent microscopy and the cell viability stains calcien-AM (live stain) and ethidium homodimer (dead stain) and counted objectively using an in house cell counting software. Cell count values were standardized by dividing the count by the area of the tissue. Glycosaminoglycan (GAG) stability was determined based on a DMMB assay and Collagen stability was determined based on an HP assay.

Results:
All 0.1 MPa explants had higher cell density than the 1 MPa explants in both static and diurnal loads after 3 days subjectively (Figure 1) and objectively (Figure 2). All diurnal loads were higher than their static counterparts (Figure 2). HP and GAG values were significantly different only between the 0.1 MPa and 1 MPa loads in the annulus fibrosus (Figure 3).

Discussion:
To our knowledge, this is the first study to investigate the effects of a static and diurnal load on IVDs for 3 days. There were marked differences between HP, GAG and cell density values between the 0.1 MPa and 1 MPa loads in the annulus fibrosus (Figure 4). This data suggests that a 0.1 MPa load preserves the viability, and tissue integrity better than a 1 MPa load. Further research is warranted to evaluate the effect of a cyclic load upon whole organ IVDs. Furthermore, both a longer culturing period and a more diverse loading parameter should be investigated. It appears that a loading parameter may increase the ability to preserve viability in whole organ IVDs.

**Significance:** A low loading parameter may permit for the successful culture of whole organ IVDs over time. This success would enable for significant study into the causes of and potentially cures for IVD degeneration. This information could help evaluate the effects of mechanics and nutrients on the health of IVDs.

**Figure 2: Annulus Fibrosus Viability**
- A= Control (Day 0)
- B= 0 MPa (Day 3)
- C= 0.1 MPa Static (Day 3)
- D= 1 MPa Static(Day 3)
- E= 0.1 MPa Diurnal (Day 3)
- F= 1 MPa Diurnal (Day 3)
Figure 3

- Viable Cell Density
  - Significant differences between 0.1 Diurnal and 1 Diurnal p=.05
  - Approaching Significant differences between 0.1 Static and 1 Diurnal p=.06
  - Significant differences between 0.1 Diurnal and 1 Static p=.002
  - Significant differences between 0.1 Static and 1 Static p=.009

Figure 4

- Significant differences between 0.1 AF and 1 AF HP p=0.05
- Significant differences between 0.1 AF and 1 AF GAG p=0.002

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