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ORIGINAL ARTICLE

Characterization of an in vitro intervertebral disc organ culture system

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Abstract Intervertebral disc organ culture has the capacity to control mechanical and chemical boundary conditions while keeping the tissue largely intact, and allowing interventions that would be impossible or unethical on animal studies. Recent studies on ex vivo organ culture has mostly involved small animals, or been limited to development and validation studies. In this study, bovine caudal discs were used. The large animal model design ensures that sufficient tissue is available for measurement of multiple dependent variables on the same disc, and a similar aspect ratio, diffusion distance, composition and rate of proteoglycan synthesis to human lumbar discs. The first goal of this study was to refine a set of dependent variables capable of characterizing the response of the intervertebral disc to culturing and to develop a technique to measure cell viability in all three regions of the disc. The second goal was to use these variables to compare static and diurnal loading as a method of maintaining intervertebral disc structure, composition, and cell metabolism similar to the in vivo state. Static (0.2 MPa) and diurnal loading (0.1 and 0.3 MPa alternating at 12 h intervals) were applied and intervertebral discs were examined after 4 or 8 days with dependent variables including changes in geometry (disc height and diameter), composition (tissue water content, tissue proteoglycan content and proteoglycan content lost to the culture media), cell viability and metabolism (proteoglycan synthesis). Results indicate that there was a decrease in disc height and water content after culture regardless of culture duration or loading condition. Cell viability significantly decreased with culture duration in the inner annulus and nucleus; however, a significant reduction in cell viability for the diurnal versus static loading condition was only observed after 8 days in the nucleus region. No significant differences were seen in viability of the outer annulus region with time, or in any loading groups. We conclude that our system is capable of keeping bovine caudal discs alive for at least 8 days without significant changes in GAG content, or cell metabolism, and that static loading was slightly better able to maintain cell viability than diurnal loading. This system offers promise for the future studies on large intervertebral discs requiring measurements of multiple mechanical and biological dependent variables on the same tissue.

Keywords Intervertebral disc · Tissue culture · Mechanobiology · Cell viability · Bovine coccygeal disc

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Introduction

Understanding the relationships between chemical and mechanical factors and intervertebral disc structure, composition and metabolism are critical to understanding disc mechanobiology in health and disease. Organ culture studies of the intervertebral disc provide a high level of control over mechanical and chemical boundary conditions while maintaining in situ tissue mechanics, thereby offering a promising model system. However, organ culture of intervertebral discs has proven to be a complex undertaking due to tissue

inhomogeneity and the high swelling potential [31] of the nucleus pulposus, and as a result relatively few studies have been published on this topic. Previous studies on cultured intervertebral discs have used a variety of tissue sources including rabbit [6, 16, 29], murine [2], rat [18, 25, 26] and bovine [17, 23]. Bovine discs have been considered a prime candidate for intervertebral disc mechanobiology studies because of their large size (area around 430 mm^2, volume around 3,376 mm^3) and similar aspect ratio, diffusion distance and resting pressure (0.2–0.3 MPa) to human lumbar discs [24]. Bovine caudal discs have also been found to be compositionally similar to human lumbar discs, with comparable hydration, collagen and proteoglycan profiles and similar rate of proteoglycan synthesis [7, 24].

Intervertebral disc swelling can be reduced or eliminated in a variety of ways including retaining endplates and adjacent soft-tissues [2, 13, 16, 18, 26, 29], embedding in alginate gel [6], adding hyperosmotic solution [31] and/or applying an external load [17, 23]. Recent studies have suggested that post mortem capillary bud clogging can impair normal nutrient and fluid flow through the endplates, suggesting that pre-mortem use of anti-coagulants or post-mortem removal of endplates may be necessary to retain in vivo transport, mechanical properties and cell viability [17, 32]. In the absence of the vertebral endplates, the application of an external load has been shown to be effective in constraining disc swelling in bovine discs [17, 23], and was the method of choice in this study because it was considered to most closely mimic the physiological loading conditions. Ohshima et al. [23] developed a chamber capable of applying an external load for bovine tail intervertebral disc culture. Their model was used to study the effect of static loading on the time course of matrix synthesis rates for up to 8 h. Lee et al. [17] developed an organ culture chamber to study young bovine tail intervertebral discs under an applied static load for 1 week, and reported that retaining vertebral endplates of intervertebral discs in culture obstructed transport and resulted in a marked decrease in cell viability after 1 week. In contrast, culturing discs without vertebral endplates was able to maintain nucleus pulposus cell viability, yet there was a significant drop in biosynthetic activity within 2 days of culture that may have been associated with the static baseline loading protocol, since static compression is known to down-regulate biosynthesis in cartilaginous tissues [5, 27].

It is still unclear whether applied static mechanical loading is optimal at maintaining the intervertebral disc in its in vivo state, or if cell and tissue health would be improved by applying loads diurnally to the disc. Furthermore, most studies on disc organ culture have either focused on cell viability in the nucleus pulposus or have not investigated viability in a region specific manner, and we believe developing a methodology to evaluate potential differences in cell viability in situ is a priority. The first goal of this study was to refine a set of dependent variables capable of characterizing the response of the intervertebral disc to culturing and to develop a technique to measure in situ cell viability in all three regions of the disc. The second goal was to use these variables to compare static and diurnal loading as a method of maintaining intervertebral disc structure, composition and cell metabolism similar to the in vivo state.

Materials and methods

Sixty intervertebral discs from the tails of 18–24 month old beef cattle were obtained from a local abattoir. Three intervertebral discs, corresponding to caudal levels c2–3, c3–4 and c4–5, were dissected from each tail under sterile conditions within 4 h of slaughter. In order to maintain transport through the endplate route, dissection included removal of vertebral endplates from the intervertebral disc using a straight edge razor blade. Following dissection, discs were rinsed in Tyrode's Balanced Salt Solution (TBSS) containing 0.3 μl/ml penicillin/streptomycin and 0.1 μl/ml fungizone (Invitrogen, Carlsbad, CA).

Discs were assigned to one of the five groups (Table 1) examining static versus diurnal loading for periods of 4 and 8 days as well as day 0 (fresh) control. The anatomic level of the disc is known to affect cell metabolism and tissue composition [33] so equal numbers of each disc level were assigned to each group. Discs assigned to culture groups were placed into a custom built organ culture chamber based on previous designs [17, 23]. The chamber consisted of a

Table 1 Test groups in this study

Group	Duration (days)	Load (Mpa)
Control (n = 20)	Day 0 control	–
Four-static (n = 10)	4	0.2
Eight-static (n = 10)	8	0.2
Four-diurnal (n = 10)	4	0.1/0.3[a]
Eight-diurnal (n = 10)	8	0.1/0.3[a]

Group designations indicate number of culture days (four or eight) and loading protocol (diurnal or static). The number of discs assigned to each group is indicated in parentheses

[a] Load switched every 12 h with the first and last 12 h held at 0.2 Mpa

top loading piston and bottom cup, both with machined ports to allow for influx and efflux of the culture medium. Sintered steel platens were attached to the loading surfaces of the culture chamber, which were then separated from the intervertebral disc by dialysis membrane (MW cut-off 12–14 kDa, Fisher Scientific, Pittsburgh, PA) to prevent excessive interdigitation of the tissue. Silicone tubing was attached from the chamber influx and efflux ports to 50 ml tubes containing culture media consisting of DMEM (4.5 g/l glucose, 110 mg/l sodium pyruvate with L-glutamine), supplemented with 100 units/ml of penicillin/streptomycin, 0.1 mg/ml gentamicin, 0.75 mg/l fungizone, 0.02 M HEPES buffer, 50 μg/ml ascorbic acid (Invitrogen, Carlsbad, CA) and 10 ml/l FBS (Atlanta Biological, Atlanta, GA). When needed, pH was adjusted to 7.4 with small quantities of sodium bicarbonate (less than 0.4 g/l). Constant media circulation was accomplished using gravity to feed the culture medium into the chamber by raising the tubes containing culture medium above the level of the chamber, and using a peristaltic pump attached to remove the medium from the chamber at the rate of 1.1 ml/min. Chambers were housed in an incubator at 37°C and 5% CO_2 and each disc was continuously bathed in media which was replaced every 2 days.

All groups, with the exception of the day 0 control, were initially confined with an applied stress of 0.2 MPa for 12 h, after which diurnal loading was simulated by applying 0.1 and 0.3 MPa in 12 h shifts and samples undergoing static loading continued under 0.2 MPa. Applied loads were returned to 0.2 MPa in the diurnal group for the final 12 h of culture to allow for final steady state comparisons between groups. Applied forces ranged from about 30 to 55 N per 0.1 MPa, and were adjusted based on the initial cross sectional area of the disc.

Structural parameters assessed included changes in intervertebral disc diameter and height. Initial height and diameter measurements were obtained using two to three caliper measurements in each dimension, recorded prior to the start of the culturing process and immediately followed culture termination.

Proteoglycan content released to the culture media and in the three intervertebral disc tissue regions (outer annulus—OA, inner annulus—IA and nucleus pulposus—NP) were assessed using the DMMB colorimetric assay [9]. Aliquots of culture media were centrifuged at 10,000 rpm for 3 min prior to the application of the DMMB assay. Tissue samples were dissected, weighed and lyophilized to obtain a dry tissue weight. The lyophilized samples were then digested first with collagenase (0.5 mg/ml at 37°C for 24 h) followed by proteinase-K (3 ml of 1 mg/ml solution added in 0.5 ml aliquots every 4 days, incubated at 57°C, Sigma Aldrich, St. Louis, MO). The DMMB assay was performed on both the collagenase and proteinase-K supernatants.

Cell metabolism was assessed using the ^{35}S incorporation assay [12]. Immediately following culture termination, sections of intervertebral disc tissue from each of the tissue regions (OA, IA, NP) were dissected, weighed and placed in 2 ml of culture medium without FBS containing 2.5 μCi of ^{35}S (Perkin-Elmer, Boston, MA) and brought to an approximate osmolarity of 400 mOsm by the addition of 1.5% v/v 5 M NaCl and 0.4 M KCl. Samples were incubated for 6 h at 37°C and 5% CO_2, after which they were removed from the radiolabel medium and digested with proteinase-K (0.5 ml of 1 mg/ml at 57°C). Radioactive media was stored for each tissue sample to allow for later normalization. After digestion, non-incorporated radiosulfate was removed by exhaustive dialysis against distilled water using a custom made microdialysis system. Radioactivity of samples was measured using a scintillation counter, and was normalized to incubation media radioactivity and tissue sample dry weight. To minimize potential artifacts due to GAG leaching that may have occurred during the radiolabel incubation step, the sample dry weight was calculated based on measurements of specimen wet weight and water content of paired tissue samples that were taken prior to incubation.

Cell viability was examined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St Louis, MO) to stain vital cells through the formation of precipitate by active mitochondria, and ethidium homodimer-1 (Invitrogen, Carlsbad, CA) to stain the DNA of non-vital cells with compromised nuclear envelopes. Tissue sections approximately 10 mm by 5 mm were dissected through the disc in the sagittal plane and placed into a TBSS solution containing 1 mg/ml MTT and 1 μM ethidium homodimer-1. After a 2-h staining period, samples were removed from the stain solution and placed on a shaker in TBSS for 10 min to remove excess dye. The tissue was then frozen in isopentane floated in liquid nitrogen and stored at –80°C until sectioning on a cryotome. Five 10 μm thick sections were taken for each tissue region (OA, IA and NP) beginning at the tissue surface and every 250 μm thereafter. For each of the 15 resulting slides, representative images were captured at 20× magnification (Zeiss axiocam, Zeiss, Thornwood, NY), first under fluorescent light to capture cells stained with ethidium homodimer-1 (Rhodamine filter: ex/em of 546 nm/617 nm) and then under

bright field light to capture precipitate formed with MTT by vital mitochondria. The two images were merged, and each section was assigned an average score based on two blinded observers who independently ranked the merged images on a scale of 1–5 based on relative cell death (1 = all live, 2 = mostly live, 3 = half live, 4 = mostly dead, 5 = all dead). Reference images for each score were also provided to the observers for comparison.

A one-way ANOVA was used to evaluate the effects of loading groups (control, 4 day diurnal, 4 days static, 8 day diurnal, 8 day static) on changes in disc diameter, height, volume and bulk weight due to culturing, water content, GAG content in the tissue, GAG content in the media and ^{35}S incorporation. A Kruskal Wallis one-way ANOVA was used to analyze the effect of loading group on cell viability. A Fishers PLSD post-hoc test was used to detect differences between groups with a significance level of $P < 0.05$ for all tests.

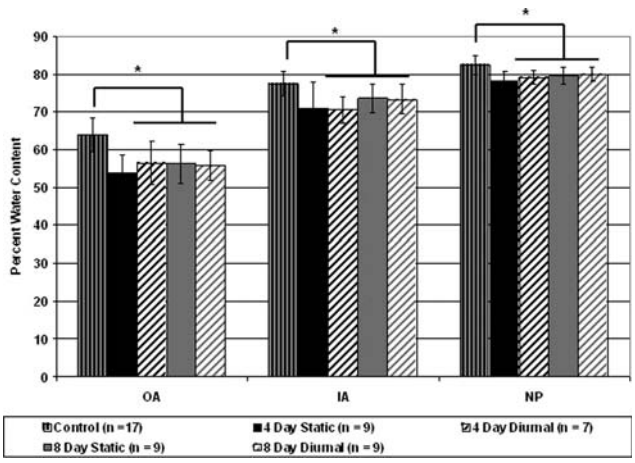

Fig. 1 Average ± SD percent water content by disc region for each of the testing groups (*OA* outer annulus, *IA* inner annulus, *NP* nucleus pulposus). All testing groups were significantly ($P < 0.05$) different relative to control (indicated by *asterisks*). *Solid bars* represent static loading groups and diagonal striped groups represent diurnal loading groups. Of the loading groups bars containing *black* are 4 days sample and *gray* represent 8 days sample

Results

For all culture groups, changes in geometry relative to day 0 are shown (Table 2). Disc diameter increased on average 14.5%, with the greatest increase in the 8 day static group and the smallest change in the 4 day diurnal group, although no statistically significant differences were observed between groups. Disc heights decreased on average 42.4%, with the greatest decrease seen in the 8 day diurnal and the smallest in the 4 day static, although again no significant differences were noted between groups. Bulk weights decreased an average of 16.7%, with significant differences noted between the 4 day diurnal and 8 day diurnal groups, and between the 4 day static group and both 8 day groups.

Tissue water contents significantly decreased in loaded culture groups relative to controls in all tissue regions ($P < 0.05$); however, no significant differences were noticed between culture groups. The largest decrease in water content was seen in the outer annulus (OA) with an average decrease of 8.2% in test groups relative to controls. The inner annulus (IA) and nucleus pulposus (NP) had a smaller decrease in water content, with average decreases in test groups of 5.2 and 3.3% relative to controls, respectively (Fig. 1).

A detectable quantity of GAG was released to the media in all test groups. Less proteoglycan release was observed in the 4 day static group (0.83 ± 0.03%) than all other groups; however, no significant difference was observed between any of the test groups. Average GAG loss to the culture media for all groups was 0.13% of the initial tissue wet weight (Fig. 2).

Average GAG content in diurnal load groups was slightly larger than static load groups in each of the three tissue regions; however, no significant differences were noted between any of the test groups or between any test group and controls (Fig. 3). The rate of sulfate incorporation was also not significantly different between any test groups, or between test groups and controls (Fig. 4).

Table 2 Percent diameter, height and bulk weight change for each test group during the culture duration

Group	Percent of diameter change ± SD	Percent of height change ± SD	Percent of bulk weight change ± SD
Four-static	14.56 ± 4.65	–37.12 ± 13.63	–10.86 ± 7.14^{++}
Four-diurnal	12.57 ± 4.67	–40.92 ± 12.08	–15.37 ± 6.91*
Eight-static	15.63 ± 6.17	–44.87 ± 7.22	–18.34 ± 5.07^{+}
Eight-diurnal	15.22 ± 4.43	–46.64 ± 8.45	–22.22 ± 7.33$^{*, +, ++}$

For all test groups, diameters increased while heights and bulk weights decreased. Group designations indicate number of culture days (four or eight) and loading protocol (diurnal or static). Common symbols indicate a significant difference ($P < 0.05$)

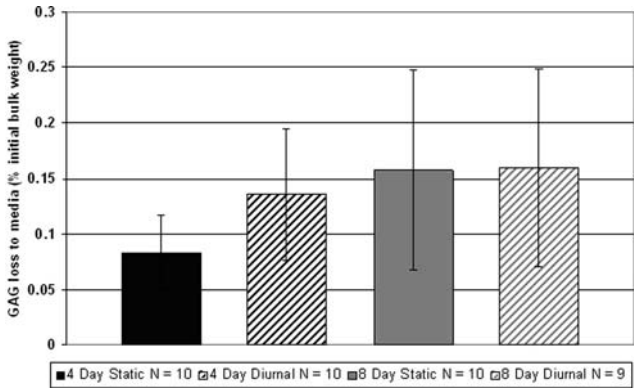

Fig. 2 Average ± SD GAG loss to the culture media over the course of the culture period. Values are expressed as a percentage of initial disc bulk weight. No significant effect of loading groups was detected. Bars have similar patterns as in Fig. 1, with the additional control specimen being denoted with *vertical bars*

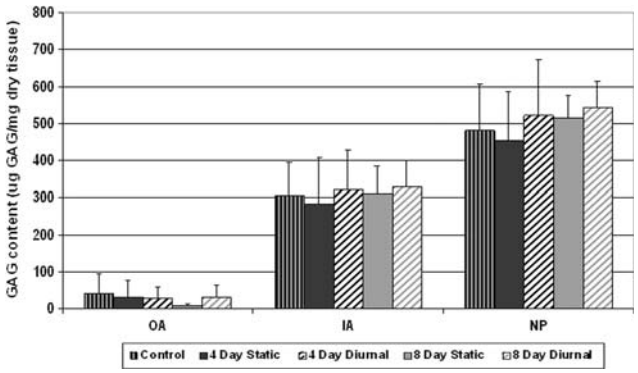

Fig. 3 Average ± SD GAG content by tissue region at the end of each testing period (*OA* outer annulus, *IA* inner annulus, *NP* nucleus pulposus). No significant effect of loading groups was detected

Tissue samples stained for viability assessments contained both live staining cells (black) and dead staining cells (white) (Fig. 5). Cell viability in all regions of control tissue scored approximately 2, ranking it as 'mostly live' (Table 3). Samples dissected and cultured for 8 days were ranked less than 3.06 in all tissue regions under a static load and in the OA and IA under a diurnal load. However, a decrease in cell viability in the nucleus region was noted in the 8 day diurnal samples, with an average score of 3.26 ± 0.53, or slightly less than half live. Regionally, a significant effect of time was seen in the IA, with significant loss of viability found between 4 and 8 days of culture, and between controls and 8 days of culture, regardless of loading protocol ($P < 0.05$). In the NP, a significant effect of loading protocol was observed, with significant differences between 4 days of static and 4 days of diurnal loading, and between 8 days of static and 8 days of diurnal loading ($P < 0.05$). Time was also found to be a significant factor, with significant differences between 8 days of diurnal loading and controls, and 8 days of diurnal loading and 4 days of static loading, mainly emphasizing a substantial loss of viability in the diurnal loading groups over the 8 day culture period. No significant differences were seen between any test groups in the outer annulus.

Discussion

We applied and refined protocols for a series of dependent variables that characterized the intervertebral disc in organ culture under static and diurnal loading. In addition, we compared freshly dissected tissue to cultured tissue to examine the relative effects of static loading and diurnal loading on cell and tissue health. Our culture system maintained cell metabolism and GAG content, and generally maintained cell viability in bovine caudal discs for at least 8 days. Cell metabolism was maintained with both static and diurnal loading conditions; however, static loading resulted in slightly better cell viability and was considered to be a preferable baseline loading condition.

The height of the intervertebral discs and the tissue water contents decreased with culturing relative to freshly dissected control tissue. While large axial deformations were noted, when put in the context of the literature [1], it is likely that this loss of disc height may have been associated with a return to more physiological disc height and water content after post-mortem swelling, and not that our choice of loading conditions were too high. Evidence for this can be found in previous studies performed only 1–2 h post mortem which have shown bovine nucleus pulposus tissue water contents closer to the results found in this study after culturing [7]. In addition, an in vivo rodent study reported an increase in caudal disc height resulting from the loss of muscle tetanus, which is likely accompanied by an increase in hydration which was restored with the application of static load [20]. Finally, the absence of a significant difference in disc heights and tissue water contents between 4 and 8 days of culture indicates that most of the height and water loss occurs early in culture, and does not appear to be influenced by culturing past 4 days, signifying that the height loss is not being caused by continuous creep of the intervertebral disc.

GAG loss to the media was lower in the 4 day static group than any other group, while the difference between the 8 day diurnal and 8 day static group was

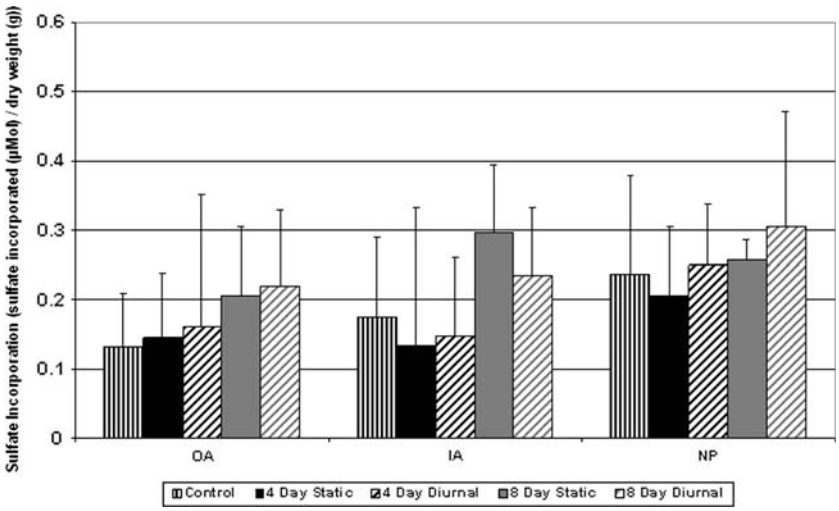

Fig. 4 Average ± SD sulfate incorporation rates normalized to tissue wet weight for all testing groups (*OA* outer annulus, *IA* inner annulus, *NP* nucleus pulposus). No significant effect of loading groups was detected

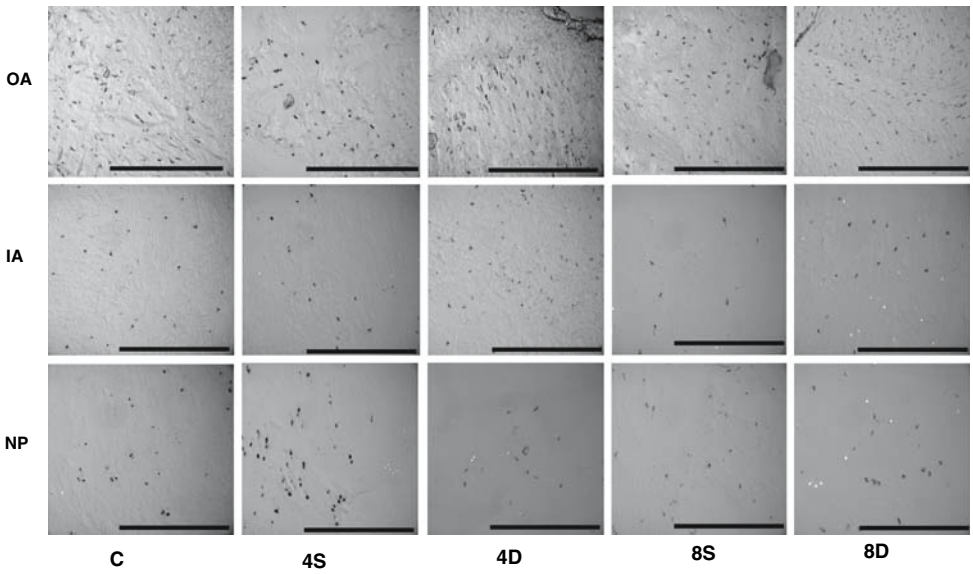

Fig. 5 Representative viability images at ×20 magnification (*black* alive, *white* dead). Scale bar in black equals 400 μm. *Columns* represent test groups [*C* control, numbers correspond to days in culture (four or eight) and *S* and *D* represent static and diurnal loading, respectively], and rows represent tissue regions (*OA* outer annulus, *IA* inner annulus, *NP* nucleus pulposus)

Table 3 Viability score for each test group by tissue region

Group	Outer annulus (OA)	Inner annulus (IA)	Nucleus pulposus (NP)
Control	1.77 ± 0.28	$1.95 \pm 0.78^{*,\ ++}$	$1.83 \pm 0.30^{\ddagger}$
Four-static	1.68 ± 0.29	$1.66 \pm 0.25^{+,\ \S}$	$1.57 \pm 0.48^{+,\ ++}$
Four-diurnal	1.66 ± 0.92	$1.59 \pm 0.82^{\ddagger,\ £}$	$2.73 \pm 1.38^{+}$
Eight-static	2.37 ± 1.23	$3.01 \pm 1.09^{++,\ \S,£}$	$2.38 \pm 1.25^{*}$
Eight-diurnal	2.12 ± 0.55	$2.98 \pm 0.59^{*,\ +,\ \ddagger}$	$3.26 \pm 0.53^{*,\ ++,\ \ddagger}$

Group designations indicate number of culture days (four or eight) and loading protocol (diurnal or static). Values are presented as averages ± SD. Common symbols indicate a significant difference ($P < 0.05$)

negligible. The difference may be explained by considering that there is a set amount of GAG that is released by disruption of the collagen matrix during dissection. The release of this GAG from the tissue is facilitated through the diurnal loading process, in which water contents increase and decrease when the intervertebral disc regains mechanical homeostasis with the changing applied load.

A decrease in cellular metabolism with static loading has previously been seen in the cultured intervertebral disc [13, 17]; however, no significant changes in cell metabolism were found after culturing under a

static load in this study. Possible reasons for these differences may be that the animals in our study were older (18–24 months) than in the study by Lee et al. (3–6 months old) [17] and the difference in loading conditions in which mechanical compression was used in this study, while Haschtmann et al. [13] applied osmotic loading. Previous organ culture work has also indicated a loss of metabolism within the first week [26]; however, that study was performed in the absence of mechanical load which is known to affect disc cell metabolism [15]. That baseline static and diurnal loading maintain levels of sulfate incorporation are consistent with in vivo findings using a rat tail model that mRNA expression of aggrecan maintained in response to immobilization [21]. This finding also provides further support to the hypothesis that intervertebral disc cell metabolism has thresholds of loading above and below which cell metabolism is affected [14], since the intervertebral disc literature reports moderate levels of static compressive load or strain can induce an anabolic response for intervertebral disc cells in vitro [28].

The method of measuring cell viability in all three regions of the disc is considered an important feature of this study, providing the capability of identifying localized areas of viable or non-viable cells in proximity to tissue features such as collagen tissue matrix disruptions. Initial attempts to use a fluorescent live and dead cell staining kit (LIVE/DEAD, Molecular Probes, Eugene, Oregon) were abandoned due to a combination of non-specific staining of the Calcein-AM (LIVE) dye resulting in difficulties locating specific areas of live cell staining in the outer annulus and the outer part of the inner annulus, consistent with previous work [17]. Extensive protocol development was performed attempting to remedy this problem, including changing dye concentrations, varying freezing methods and fixing tissues immediately after staining. The final choice for live cell marker was MTT, previously used for cartilage viability studies [8] because it was easily visible in the dense outer annulus region.

Average viability scores in this study should be interpreted as qualitative or semi-quantitative measurements that do not correspond to a precise viability percentage. An important distinction in cell viability between loading protocols was considered an easily identifiable change. The grading scheme was developed accordingly to examine many tissue slides and provided a representative measurement. Other investigators have more precisely quantified viability in the cultured intervertebral disc by reporting fluorescence intensity of Mitotracker Red in the nucleus pulposus [26], a method that was not used in this study because non-fluorescent cell viability makers were required for annulus regions. However, the 'mostly live' grade (≤2) obtained in this study for control samples is consistent with the finding of around 85% cell viability by Haschtmann et al. [13] using cell isolation technique (eliminating region specific differences) and with representative nucleus pulposus images presented by Lee et al. [17]. Lim et al. [18] examined in situ viability in a rat motion segment using a visible live cell marker (NitroBlue Tetrazolium) counterstained with DAPI and reported cell viability >95% after 14 days in culture, which was notably high and may be associated with their normalization technique, species differences and/or the fact that removal of vertebral endplates required to maintain bovine discs cell viability has greater co-morbidity.

It is interesting to observe the different regional response to the loading and time protocol. Where the outer annulus had no apparent changes in viability over the course of the experiment, the inner annulus tend to decrease in relation to time, and the nucleus pulposus tend to decrease in relation to an interaction between time and the applied loading protocol. Comparisons of absolute cell viability values may be impossible due to differences in techniques, yet it appears that the current system results in a more rapid decrease in cell viability relative to control tissues than for other systems [13, 18, 26] and this is most likely related to specimen size and age as the large size of bovine discs presents a greater challenge to older cells, as used in our study. Despite the loss of cell viability, there is a trend of increasing sulfate incorporation with time, suggesting that cells are adjusting to the culture environment and increasing their cell metabolism following initial harvest and placement in the culture chamber. The greatest loss in cell viability in our study was detected in the nucleus, consistent with Lim et al. [19], yet our study is one of the only one to present viability in the inner annulus for discs in organ culture, and our results suggest that viability in this region is also sensitive to loading and time, consistent with in vivo animal models.

The finding that diurnal loading provided a greater challenge to cells with a slight reduction in viability relative to static loading is consistent with Haschtmann et al. [13] who reported a decrease in cell viability in motion segments subjected to a diurnally applied hyperosmotic load after 14 days that they associated with impaired osmoregulatory capacity of native tissue after the specimen harvest procedure. In context of previous studies on nutrition and transport [11], we infer that decreased cell viability in diurnal loading was

not associated with nutritional factors but may be associated with increased fluid shear effects on the cells that were pronounced in our specimens because of the removal of vertebral endplates. A more thorough description of the region-specific relationships between mechanical loading and cell viability will help to define how specific populations of cells in their native matrix respond to loading with the potential for region-specific repair in the future.

One limitation of this study is the removal of endplates from the intervertebral disc. It has been demonstrated that the vertebral endplates deform under compressive loading for human and rat models [4, 10, 22]. While this is anticipated to affect absolute values of dependent variables measured, it is not expected to affect relative comparisons with time and loading condition. In this study, the osmolarity of the culture medium was not adjusted since we used an applied loading to contain swelling. While hyperosmotic conditions are developed in the disc partially in response to fluid exudation caused by daily diurnal mechanical loading [3, 30], future investigation of interactive effects of mechanical and osmotic loading is warranted and may be addressed using the current culture system.

In this study, significant differences were only observed between static and diurnal loading in the case of cell viability, where a slight decrease in nucleus pulposus viability was observed with diurnal loading. Significant differences were observed between freshly dissected tissue and cultured tissue in some cases of cell viability, in water content, and in a comparison of final disc height after culturing and initial freshly dissected disc height.

Conclusions

This study developed and implemented a large animal organ culture system and determined the most appropriate baseline loading conditions based on results using several dependent variables that thoroughly characterized disc structure, composition and metabolism. Methods were developed for measurement of cell viability in all disc regions. Results indicated that static and diurnal loading conditions both maintained GAG content and cell metabolism in the intervertebral disc organ culture; however, static loading resulted in the best cell viability and is considered a preferable baseline loading condition. Alterations in disc geometry and water content in all loaded groups were observed consistent with previous studies, and we speculate this may be due in part to post-mortem swelling due to muscle relaxation followed by a return to more physiological hydration levels under baseline loading conditions. This system may be applied in future studies to examine region-specific interactions between applied mechanical or chemical conditions on tissue composition, structure, cell viability and metabolism.

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