INTRODUCTION

The intervertebral disc plays the essential biomechanical roles of supporting load and permitting motion in the spine. Damage and degeneration of the disc affects its water and proteoglycan contents as well as its elastic and viscoelastic behaviors. In vivo MRI studies reported loss of water content in response to sustained loading in all disc regions but the outer annulus [1]. There is limited understanding of the time-course for water loss and viscoelastic creep in a controlled in vitro environment where inferences may be made regarding volume loss and mechanisms of viscoelasticity. The purpose of the current study is to test the hypotheses that:
1) There is no difference in height loss and fluid (volume) loss of discs loaded in compression under cyclic (0.15-1.0 MPa) and static conditions with the same time averaged magnitude (0.57MPa).
2) After initial disc bulge, tissue water loss is directly proportional to height loss under static loading.

MATERIALS AND METHODS

One hundred twenty six caudal motion segments from twenty one 16-week old female Wistar rats were used in this experiment. Rats were obtained within a half hour of being euthanized. Skin and soft-tissues were removed from the tails, six adjacent motion segments were harvested from tail, and vertebral bodies of each motion segment were potted in aluminum tubes using cyanoacrylate. Motion segments were frozen in liquid nitrogen and wrapped in phosphate buffered saline (PBS) soaked gauze and plastic to prevent dehydration during storage. All specimens underwent one of five loading protocols (Table 1) using custom grips and a force-controlled test protocol on an axial testing machine (Enduratec ELF-3200, Bose Corporation, Minnetonka, MN)[2].

Table 1. Each motion segment was assigned to 1 of 5 loading groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Static I</th>
<th>Static II</th>
<th>Static III</th>
<th>Cyclic Mid-Static</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load (MPa)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>.57</td>
</tr>
<tr>
<td>Duration</td>
<td>9 min.</td>
<td>90 min.</td>
<td>900 min.</td>
<td>90 min.</td>
</tr>
</tbody>
</table>

After loading, each disc was frozen and separated into nucleus pulposus and annulus fibrosus sections. Tissue sections were immediately placed in pre-weighed vials and wet tissue weights were measured. Specimens were subsequently lyophilized (-60°C for 24 hours) to obtain dry tissue weights. The % water content, and % water loss from loading were calculated.

The creep behavior of the motion segment was fitted using the stretched exponential model, as previously described [2];

\[ d(t) = d_e + (d_i - d_e) e^{-\frac{t}{\tau}} = d_i + (d_i - d_e) e^{-\frac{t}{\tau}} e^{-\gamma} \]

where \( d_i \) and \( d_e \) are the initial and equilibrium displacements, and \( \tau \) and \( \gamma \) are the time constant and stretch parameter, respectively.

RESULTS

Both disc height loss and water loss under static compressive loading were time-dependent until equilibrium level was achieved within 900 minutes. The decrease in disc height with time was significant between the three 1.0 MPa static loading groups (Figure 1a, p<0.01), Water contents of the annulus fibrosus and nucleus pulposus also decreased over time under compressive static loading at 1.0 MPa, though groups were not significantly different. Amongst the 90 minute duration groups (Figure 1b), Cyclic loading resulted in height loss that was significantly greater than Mid-Static (p<0.01), but not significantly less than Static II loading at 1.0MPa (p=0.2). For all loading groups, % water loss was greater in the nucleus pulposus tissue than in the annulus fibrosus. (p<0.01).

Transient behavior in the three 90 minute groups was compared using mean creep displacement curves, as fitted using the stretched exponential model (Figure 2). For the entire time period, average displacement for the Cyclic loading is significantly higher than that of the Mid-Static condition, and the cyclic peaks are nearly equal to the creep displacement of the 1.0MPa static condition.

DISCUSSION

Contrary to our first hypothesis, the cyclic loading regimen resulted in a significantly greater decrease in disc height and annulus fibrous water content than did static loading at the root mean square value (0.575 MPa – Mid-Static group). The most important finding of this study was that cyclic loading from 0.15-1.0 MPa resulted in disc height and water losses that were similar to static loading at 1.0MPa over the same time span. These results indicated that the peak compression magnitude of cyclic loading at a frequency of 1 Hz dominated the height change and water loss rather than the time average magnitude. We speculate that this is associated with differences in creep and recovery rates of the disc.

In agreement with our second hypothesis, a nearly linear relationship between height loss and water loss suggested that creep occurring after 9 minutes was the result of volume change (water loss). Interestingly, more water was lost relative to the height change in the nucleus than in the annulus suggesting some water redistribution. The time-dependent water loss is consistent with an initial elastic compression (and associated disc bulge) followed by a reduction in disc volume with time. Both main findings have important implications for mechanobiology studies of the disc and in the definition of safe loading protocols for the disc.

REFERENCES


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