Effects of Enzymatic Digestion on Mechanics of In-Plane Annulus Fibrosus Lamellae

Isaacs, JL; Bonfiglio, D; Gidvani, S; Vresilovic, E; Marcolongo, M

INTRODUCTION
The annulus fibrosus of the intervertebral disc (IVD) is composed of ~25 lamellae comprising unidirectional collagen fibers with about 40 collagen bundles/ lamella [1]. Consecutive lamellae (300 μm thick) run in opposite directions with fibers at an angle of 60° to the long axis of the spine. Annular tears, which are an early indication of disc herniation, are related to lower back pain but their relationship to IVD degradation and load is currently unknown [3]. Herniation of the lumbar IVD is characterized by a radial displacement of nuclear or annular material and can result due to a traumatic event, mechanical overload under unfavorable conditions, or the degenerative process of aging [4]. Concurrently, the amounts of collagen and elastin within the IVD change with age; Collagenase and Elastase enzymes mimic degeneration of the AF by removing collagen and elastin from the tissue, respectively. To understand the mechanical consequences of these degenerative changes, we have developed a single lamellar model under different degenerative conditions to test annulus lamellar biomechanics in plane to the collagen and elastin fiber directions using a micro-mechanical test protocol and post processing micro-strain analysis.

METHODS
Single lamellar (150 μm thick) human lumbar annulus fibrosus (AF) samples were cut using a Leica 3050S cryostat with a tungsten carbide blade. The samples were placed into four groups and digested using the following protocol.

<table>
<thead>
<tr>
<th>Group</th>
<th>pH</th>
<th>Enzyme</th>
<th>Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>7.4</td>
<td>—</td>
<td>1X PBS</td>
</tr>
<tr>
<td>PG</td>
<td>8.0</td>
<td>Chondroitinase ABC (0.125U/5mL)</td>
<td>0.02% BSA, 50mM TRIS, 60mM Sodium Acetate</td>
</tr>
<tr>
<td>COL</td>
<td>7.4</td>
<td>Collagenase (10U/5mL)</td>
<td>50mM TES, 2mM CaCl₂</td>
</tr>
<tr>
<td>ELA</td>
<td>8.5</td>
<td>Elastase (10U/5mL)</td>
<td>0.1M TRIS</td>
</tr>
</tbody>
</table>

An initial experimental group was tested using only buffers to determine if there was an effect on the mechanical properties. It was determined that the pH of the buffer had an effect on the mechanical properties. Therefore, the enzymatically-digested groups would be compared to their respective control (buffer only) instead of the 1X PBS control group. Samples were pre-strained to e = 0.1 then with an average strain rate of 0.02 mm/sec were tested until failure. Micromechanical stress-strain curves were compiled and concurrent video streams captured morphological damage mechanics.

RESULTS

![Figure 1: The varying buffers affect the mechanical properties of the tissue. Effect of enzyme will be compared to respective buffer control.](image)

![Figure 2: The effect of buffers and enzymes on the mechanical properties of individual lamellae of the AF. *p<0.05 from 1X PBS.](image)

DISCUSSION

The absolute values of the mechanical properties for all the groups, enzyme and buffer are lower than that of the control group (1X PBS). The buffers have more dramatic effect on the properties than the enzyme and buffer combinations. The effect of the enzyme on the mechanical properties may be masked to some extent by the buffer conditions on the tissues. The collagenase-treated group reduced the ultimate tensile strength by 75% from the non-degraded group (1X PBS), indicating the importance of collagen to the tensile strength of the annulus fibrosus. Both the Elastase-treated and Chondroitinase ABC-treated groups resulted in a reduction of the ultimate tensile strength by about half. This suggests that elastic fibers function to guide and restrain the deformation of the collagen matrix and that on their removal, the collagenous elements may play an even more dominant role in the tissue mechanics. A limitation in the sample preparation allows the introduction of edge effects into the mechanical properties [8], however, the properties described here provide insights into the intralamellar mechanical behavior, which may allow the further understanding of alteration in mechanics seen with disc degeneration leading to herniation.

REFERENCES


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