The Effects of Sustained Administration of Growth Factors on Traumatized Discs Using Adult Male Rats
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INTRODUCTION
The current modalities of treating symptomatic degenerative disc disease are either conservative non-surgical or surgical modalities. Non-surgical modalities include improving body posture and back muscle strengthening through physical therapy. Surgical treatment includes removal of the disc material and intervertebral fusion, or artificial disc replacement, which is still in experimental phases in the United States. However, none of these modalities is a true cure for the degenerative process. Ideally, the best treatment would be preventing the progression of degeneration. An understanding of the mechanisms involved in intervertebral disc disease is crucial to develop new methods to prevent disc degeneration. The goal of our research is to shed light on the biochemical aspect of disc degeneration and identification of growth factors that may slow or stop the degenerative process. It is important to determine the impact of the growth factors long-term on degenerated discs, and the appropriate timing of the delivery and therapeutic dose of growth factor that can lead to an effective treatment regimen.

WE hypothesize that continuous sustained release of transforming growth factor beta (TGFβ) and insulin like growth factor-1 (IGF-1) alone and in combination will reverse the loss of cellularity associated with degenerating discs.

METHODS
Research Design: A degenerative disc adult male rat model was developed, and a drug delivery device was used to show the capability of TGF-β or IGF-1 to be used for slowing or reversing the decline of cellularity within the degenerating disc over time. Thirty adult male Sprague Dawley rats were divided into five equal groups (n=6). Group I naive control, group II damaged disc alone, group III damaged disc + TGF carrier (2 pg/day), group IV damaged disc + IGF-1 carrier (2 pg/day), group V damaged disc + TGF-IGF-1 carrier (1 pg/day or each factor). All carriers were delivered over a 2 week period, and the discs were harvest four weeks post-surgery.

Fabrication of TCPL Microcrystals: Microcrystals of tricalcium-phosphate powder were prepared using standard laboratory procedures (Benghuzzi et al., 1988, 1990, 2006).

Surgical Defect and TCPL Implantation: A longitudinal right paramedian incision approximately one inch in length was made. The external oblique muscle was exposed. The lumbar spine was palpated through the external oblique muscles and blunt dissection was performed through the fibers of the external and internal oblique muscles. The bodies of the distal lumbar vertebrae were exposed. A 21-gauge needle was used to identify and induce trauma into the vertebrae. The targeted discs are L5-L-6 intervertebral disc (the rat has six lumbar vertebrae and the rat has six lumbar vertebrae). The disc was poked. The data is displayed in μm ± SD. (n = two animals per group).

RESULTS
Piercing the disc induced structural damage and accelerated degeneration of the annulus and nucleus without evidence of inflammation when compared with non-traumatized controls. After four weeks, animals in group II (trauma only) showed evidence of disc degeneration with the largest decrease in cell number anterior to the site of trauma. Animals treated with growth factors increased cell numbers within the posterior lateral areas when compared with sham animals (Table 1). Disc heights of both the damaged and adjacent discs were measured and compared for each group and compared with control. There were no differences in the disc height in the adjacent disc height between the groups. The damaged disc height in the sham was significantly less than all other treatment groups and control (Figure 2). The growth factors either alone or in combination were able to maintain disc height comparable to control. However, the combination of TGF+IGF appeared to have cellularity similar to control undamaged disc (Figure 3).

Table 1: Nuclei/area counts average of 4 different animals per group and 4 different views per slide.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Anterior</th>
<th>Lateral</th>
<th>Posterior</th>
<th>Lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.3 ± 0.84</td>
<td>12.0 ± 0.75</td>
<td>13.0 ± 1.05</td>
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</tr>
<tr>
<td>Sham</td>
<td>3.8 ± 0.62</td>
<td>5.8 ± 0.94</td>
<td>9.8 ± 0.99</td>
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<tr>
<td>TGF</td>
<td>7.5 ± 0.90</td>
<td>6.3 ± 0.60</td>
<td>9.6 ± 1.21</td>
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<tr>
<td>IGF</td>
<td>8.9 ± 1.06</td>
<td>8.7 ± 0.91</td>
<td>8.4 ± 0.94</td>
<td></td>
</tr>
<tr>
<td>TGF + IGF</td>
<td>9.6 ± 0.95</td>
<td>7.4 ± 0.62</td>
<td>9.0 ± 1.10</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION
Walsh et al., (2004) evaluated four different growth factors (TGF-β, IGF-1, FGF, and growth and differentiation factor-5 (GDF-5)), in a murine disc degeneration model. In their investigation the degenerated disc was injected with single or multiple injections of an equal volume of growth factor or saline (control) and compared with intact control over a 4 week period. Their results showed that only TGF-β had a stimulatory effect on the annular fibrochondrocytes compared with saline controls. The other growth factors given in multiple injections were not able to increase the population of chondrocytes in the annulus or nucleus or cartilage endplates, and multiple injections resulted in collapse of adjacent disc and inflammation. Our results show continuous delivery of growth factors can increase cellularity, with a combination of growth factors representing most similar to control.

REFERENCES

ACKNOWLEDGEMENT
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