A SIMPLE NOVEL ANNULAR PUNCTURE MODEL OF DISC DEGENERATION IN THE MOUSE TAIL

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INTRODUCTION
Animal models have long been used for studying the pathogenesis of the degenerative disc disease (DDD) and evaluating different therapeutics in regenerating the disc. Despite the small size of the disc, mouse is always a tempting model of DDD because of the widely known mouse genome and abundant molecular tools. The objective of this study is to create a simple reproducible model for studying DDD in mice, using the technique of annular puncture in the mouse tail. We observed that the punctured mouse disc undergoes rapid and progressive degeneration over a period of 12 weeks. We hope that this mouse model can be useful in future study to understand the molecular mechanisms of disc degeneration.

METHODS
With approval by the local ethics committee, the tail (caudal 4-5 level) of thirty mice aged 10 weeks were punctured under microscopic guidance using a 31G needle. The depth of puncture was carefully controlled. Radiographs were taken for each mouse at 2, 6, 12 weeks after the surgery and disc height measured. Additionally 5 mice were sacrificed at each time point: 2, 6, and 12 weeks after surgery. The punctured disc and adjacent level control discs were removed for histology (H&E, Safranin O and Alcian blue) and RT-PCR.

RESULTS
Radiographs showed progressive disc height reduction suggestive of degeneration (Figure 1). This was confirmed on the Safranin O stained histological sections (Figure 2, and Table 1). RT-PCR results (Figure 3) showed that Col1a1 expression increased from 2 weeks to 6 weeks and decreased at 12 wks. The expression of Col2a1, Sox 9 and aggrecan decreased continuously from 2 wks to 12 wks.

DISCUSSION
This is the first description of an annular puncture mouse tail model of disc degeneration. The punctured mouse disc degenerated progressively from 2 wks to 12 wks as evidenced by a loss of disc height, and disorganization of the annulus and structural changes within the growth plate. Interestingly the nucleus pulposus, which in the mouse consists of large numbers of notochordal cells, changes to a “chondrocyte-like” appearance within 2 weeks of puncture, and then progressively degenerates with reduction in proteoglycan level. While relevance to human disc degeneration is not known, this model may be useful for understanding the underlying molecular mechanisms regulating disc degeneration and methods to regenerate it.

REFERENCE
Sotashi et al. Spine 2004; 30(1); 15-24

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Figure 1 Disc height measurement

Figure 2 Safranin O staining of the intervertebral disc

<table>
<thead>
<tr>
<th>Time</th>
<th>AF Description</th>
<th>NP Description</th>
<th>Growth Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>parallel lamellae</td>
<td>highly organized</td>
<td>smooth</td>
</tr>
<tr>
<td>2 wks</td>
<td>disorganized</td>
<td>replaced by proteoglycan rich matrix</td>
<td>tilted</td>
</tr>
<tr>
<td>6 wks</td>
<td>indistinct</td>
<td>cell clusters formed</td>
<td>wavy</td>
</tr>
<tr>
<td>12 wks</td>
<td>clefts formed</td>
<td>clefts formed</td>
<td>disrupted</td>
</tr>
</tbody>
</table>

Table 1 Summary of histology

Figure 3 Gene expression analysis