Author's response to reviews

Title: Observations on morphologic changes in the aging and degenerating human disc: Secondary collagen alterations

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PDF covering letter
Response to Dr. Barry Berkovitz:

We thank the reviewer for the helpful comments; wherever possible the suggested changes have been carried out. Specific comments to Dr. Berkovitz’ comments follow:

1) The text change has been made as suggested.

2) As suggested, the ages have now been places in the main Methods section.

3) We have seen the same types of changes in lumbar, thoracic and cervical disc specimens. The text has now been modified to state that the majority of the specimens studied here were anterior portions of lumbar discs.

4) Yes, quantitative data would be a very useful part of future studies. We now have a new paragraph addressing limitations of the present study; this has been added as the next to the last paragraph of the Discussion and the text now reads:

   “A limitation of the present study is the qualitative nature of our study. Future studies would be greatly strengthened by quantitative analysis of variations of crimping periodicity in both surgical specimens and in representative sections of complete discs. Such quantitative studies are important since histologic/ultrastructural examination only focuses upon a small portion of tissue. Additional important information lacking from our study is determination of the extent to which the changes described here actually alter the biomechanical properties of the disc. We hope that our small study here will lead to more complete studies of this important topic since there is much to be learned about how disc degeneration relates to increased risk of herniation and pain.”

With respect to the question on cross-sectional diameter of collagen fibrils, yes we do see variations in this in both control and surgical specimen. A new paragraph (before the “limitations of study” paragraph now states:

   “In other ultrastructural studies of disc tissue, we have reported considerable variation in cross-sectional diameters of collagen fibrils in both territorial and inter-territorial extracellular matrix [8]. Cross-sectional diameter variability was present in 34% of control and 59% of surgical specimens which were examined. Such variations may also have a possible effect on the ability of the disc to respond to biomechanical stress.”

Your comment about the encircling matrix accumulations around the disc cells is interesting. In our previous light microscopic study, we found that these “rings” could contain Type I collagen (63-70% of the time), Type II (59-83%), and Type VI collagen (83-100%) (see our Spine publication 23:751, 1998). We thank you for the citation you provided, and agree that there may be some common ECM mechanisms between various tissues. We see these encircling rings in both our control and surgical
specimens. The same aging/degeneration changes occur in both populations, but the degree or herniation/pain caused the surgical subjects to seek orthopaedic help.

5) The two typos noted have been corrected.

**Response to Dr. Lotz:**

We thank Dr. Lotz for his thoughtful comments which were very useful in our revision. Dr. Lotz has extensive experience in disc biomechanics, and his comments were insightful. Our study was qualitative in nature, and we feel that the changes in response to the points raised in this review have improved our manuscript.

Specific responses are presented below

1) Dr. Lotz is certainly correct in pointing out the limitations of our qualitative study. We have added a new paragraph directed to these issues; it now states:

   “A limitation of the present study is the qualitative nature of our study. Future studies would be greatly strengthened by quantitative analysis of variations of crimping periodicity in both surgical specimens and in representative sections of complete discs. Such quantitative studies are important since histologic/ultrastructural examination only focuses upon a small portion of tissue. Additional important information lacking from our study is determination of the extent to which the changes described here actually alter the biomechanical properties of the disc. We hope that our small study here will lead to more complete studies of this important topic since there is much to be learned about how disc degeneration relates to increased risk of herniation and pain.”

Control specimens were derived from putative normal donors contributing tissue for research purposes to the Cooperative Human Tissue Network via the Natl. Cancer Institute. Such specimen do indeed show degenerative morphologic changes as we have previously reported in Spine 23:751, 1998 and in our Spine ultrastructural study in press. We have chosen to report our control findings as “controls” instead of “normal” because of the presence of disc degeneration in the non-symptomatic aging population. This has been documented also by Boden who found MRI evidence of substantial disc changes in both lumbar and cervical discs of asymptomatic older subjects. A comment has now been added about this, and citations to two papers of Boden et al added, in the third paragraph of the Discussion.

With respect to the comment about specimen numbers in the Abstract, this has been reworded to simplify the abstract and retain the detailed information in the Methods section of the main body of the manuscript. The abstract now reads “control (normal) donors” to help simplify this issue.
The encircling extracellular matrix lies around the disc cells. These rings consist of Types I and II collagen and type VI collagen as we have previously reported (Spine 23:751, 1998). These areas are seen in both control and surgical discs. So it is apparent that the cells are still making collagen, but its distribution is abnormal. Buckwalter and others have previously also documented this abnormal histologic feature of aging/degenerating discs. If one has these marble-like focal matrix accumulations around the cells, it disrupts the long normal course of the collagen fibers seen in healthy disc tissue.

Background:
Four to six thick sections were cut from the electron microscopy specimens and sections which were parallel to the long planes of collagen fibrils were selected. This is what was meant by “opportune sectioning planes”. This information has now been added to the main Methods section of the paper.

The Optimas software is actually graphing differences in light intensity, which reflects the collagen polarized light crimping images, in the scans of the region of interest selected in the microscopic image with the ability to designate the rotation and viewpoint of the specimen and the number of scans in the x,y plane. The images presented in Figure 4 used the same settings for rotation, sampling and viewpoint (rotation: x, 25.000; y, 1.000; sampling, x: 45, y:45, and viewpoint Z: 3000.0). Information on these specific settings has now been added to the legend of Figure 4.

Results:
“opportune sectioning planes” - this has been discussed above.

The regions of ECM encircling cells has been discussed above.

Comments about page 6: We have previously analyzed the incidence of matrix changes in our paper in press in Spine. The table below is reproduced form this paper for the Dr. Lotz’ information:

<table>
<thead>
<tr>
<th>Ultrastructural Finding</th>
<th>Control Specimens</th>
<th>Surgical Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ECM:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrous long-spacing</td>
<td>41.3%</td>
<td>36.7%</td>
</tr>
<tr>
<td>collagen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segment long-spacing</td>
<td>-0-</td>
<td>2.0%</td>
</tr>
<tr>
<td>collagen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variable collagen cross-sectional diameters</td>
<td>34.4%</td>
<td>59.1%</td>
</tr>
<tr>
<td>Markedly widened collagen fibrils</td>
<td>27.5%</td>
<td>16.3%</td>
</tr>
<tr>
<td>Spiny collagen</td>
<td>10.3%</td>
<td>2.0%</td>
</tr>
<tr>
<td>ECM layers encircling cells</td>
<td>48.3%</td>
<td>63.2%</td>
</tr>
<tr>
<td>Dense proteoglycan pools in surrounding cells</td>
<td>13.7%</td>
<td>12.2%</td>
</tr>
<tr>
<td><strong>Cellular:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inclusion bodies</td>
<td>31.0%</td>
<td>28.5%</td>
</tr>
<tr>
<td>Apoptotic nuclei</td>
<td>13.7%</td>
<td>16.3%</td>
</tr>
</tbody>
</table>
At present we do not have any other quantitative data. It would be important to be able to precisely delineate the types of changes seen throughout the disc; however, this would require considerable technical time and a large number of intact discs, both of which are in short supply in my lab at present. We have added a new paragraph at the end of the Discussion which points to the importance of such studies in the future. It reads:

“A limitation of the present study is the qualitative nature of our study. Future studies would be greatly strengthened by quantitative analysis of variations of crimping periodicity in both surgical specimens and in representative sections of complete discs. Such quantitative studies are important since histologic/ultrastructural examination only focuses upon a small portion of tissue. Additional important information lacking from our study is determination of the extent to which the changes described here actually alter the biomechanical properties of the disc. We hope that our small study here will lead to more complete studies of this important topic since there is much to be learned about how disc degeneration relates to increased risk of herniation and pain.”

Discussion:

Yes, no biomechanical tests were performed in our study, and we do not know how the specific changes, and their extent, contribute to biomechanical function. The new paragraph above acknowledges this shortcoming in our work.

The types of changes we saw have been previously reported by Buckwalter and Trout in their early ultrastructural studies of the disc. Since this is not the major focus of the present paper, and since we have cited our paper in press in Spine addressing the ultrastructure of the disc, we have not made changes in the test on this issue.