Adipose-Derived Regenerative Cell Transplantation: Evaluating intervertebral disc repair in a canine model

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Introduction: Stem and regenerative cell therapy are emerging modalities that can allow patient activity while regenerating damaged tissue (1, 2, 3). Autologous nucleus pulposus transplantation has been correlated with an increase in disc height when compared with discectomy alone at 1 year in a canine model (4). Subsequent clinical study adds that nucleus pulposus-like cells enhance matrix hydration and patient recovery following discectomy (5). Adipose tissue provides an alternative source of regenerative cells with little donor site morbidity. These regenerative cells are able to differentiate into a nucleus pulposus-like phenotype when exposed to environmental factors similar to disc, and offer the inherent advantage of availability without the need for transporting, culturing, and expanding the cells (6,7).

In an effort to develop a clinical option for cell placement and assess the response of the cells to the post-surgical milieu, adipose-derived cells were collected, concentrated, and transplanted under fluoroscopic guidance directly into a surgically damaged disc.

Materials and Methods: After IACUC approval 12 dogs, 2 years of age, were obtained. Adipose cells were harvested from the super-scapular region of the neck (scruff) and adherent cells separated, collected, and labeled with DAPI. Adipose tissue has been known for some time to contain regenerative cells in addition to fat cells (8). Three lumbar intervertebral disc levels in each dog underwent a partial nucleotomy; other levels served as non-operated controls. Levels of intervention as well as the regimen of treatment were dually randomized. Three interventions were used in this study; adipose-derived cells in hyaluronic acid (HA) carrier, HA alone, or no intervention – all deliveries were guided by fluoroscopy. Assessments were made by MRI, radiography, microscopy, RT-PCR, and ELISA.

Results: The dogs were radiographed, received MRI scans and then were euthanized by 12 months. The disc tissue was harvested from the lumbar spine in each dog. Labelled cells were assessed as viable in the tissue

Matrix composition was assessed; assays were made of aggrecan, Types I and II collagen by both RT-PCR and ELISA to assess and compare matrix regeneration. mRNA and protein from each level are presented with respect to normal values defined as the 100 percent expression Relative mRNA for specific matrix proteins

### Relative mRNA for specific matrix proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>HA</th>
<th>HA+Cells</th>
<th>No Interv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggrecan</td>
<td>100</td>
<td>85.6</td>
<td>85.6</td>
<td>100</td>
</tr>
<tr>
<td>Type I</td>
<td>100</td>
<td>87.3</td>
<td>87.3</td>
<td>100</td>
</tr>
<tr>
<td>Type II</td>
<td>97.3</td>
<td>82.8</td>
<td>82.8</td>
<td>100</td>
</tr>
</tbody>
</table>

The data were calculated with two samples t-test, comparing Control with interventions at P<0.05 and P<0.01. Statistical differences were found between the Control and each intervention at P<0.01, whereas the difference between Control and HA plus Cells was only significant at P<0.05. No significant difference could be shown between HA alone and No Intervention. These evaluations and other morphometric assessments support Cell viability follows implantation; supplementing adipose cells following injury supports regeneration; morphology was maintained; intervertebral disc height was not lost; MRI signal remained similar to native control; hyaluronic acid was insufficient to prevent disc degeneration or desiccation; lack of intervention resulted in progressive degeneration; a limited nucleotomy procedure, similar to that which would be experienced following clinical micro-discectomy, resulted in prolapsed annulus tissue into the central space of the nucleus pulposus; and no significant regeneration of cells or matrix occurred without treatment.

Discussion: This study provides evidence that cells harvested from adipose tissue might offer a reliable source of regenerative potential capable of bio-restitution. Key strengths make the case for using adipose-derived cells; first, cells can be transplanted percutaneously; and second cells survive and functionally adapt and produce appropriate matrix. The span of this study was sufficient to show that freshly isolated cells will survive the trauma associated with post-surgical inflammation. The time to treat, the cell carrier, and the ability of the cells to integrate into the disc matrix were all certainly convincing. This study provides evidence that adipose-derived regenerative cells can be transplanted at surgery using fluoroscopic guidance, and that these cells can be injected directly into the intervertebral disc with the expectation that they will remain viable and produce appropriate, tissue-specific matrix.


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