Adipose Tissue-Derived Stem and Regenerative Cell Transplantation: Intervertebral Disc Repair in a Canine Model

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Introduction
Cell intervention with autologous disc chondrocyte transplantation (ADCT) has been shown to enhance clinical recovery following discectomy (Quebec Pain disability assessment, Oswestry, and VAS)[1]. Limitations, such as expansion of autologous cells, have guided efforts to develop a process using adipose tissue-derived stem and regenerative cells (ADRCs) harvested during surgical exposure that allow cells to be returned in real time to a damaged disc. The study presented here mirrors previous work in the same model using ADCT[2].

Materials and Methods
After IACUC approval, 12 skeletally mature dogs underwent a partial nucleotomy at three lumbar levels; adjacent levels served as non-operated controls. The dogs were allowed to recover from the surgery for six weeks, at which time subcutaneous adipose tissue was harvested from the scruff of the neck and ADRCs were isolated. A fraction of ADRCs were labeled with DAPI and mixed with unlabeled ADRCs at a 1:3 ratio prior to injection in 2 dogs. The discs were randomized to receive: 1) ADRCs in hyaluronic acid (HA) carrier (Cells/HA); 2) HA only; or 3) No intervention. All cell deliveries were guided by fluoroscopy. Assessments of the discs were made by radiographs and MRI. Six dogs were euthanized at 6 months, and 6 dogs at 12 months (1 DAPI labeled ADRC per sacrifice time point). Assessments were focused on criteria that would offer clinically diagnostic potential such as disc height and MRI differentiation. Since clinical durability is a critical to successful therapeutic protocols, time-dependent analysis was a primary consideration.

Results and Discussion
An average of 12.11 ± 2.93 grams of adipose tissue was collected, yielding 3.21 x 10^9 ± 1.47 x 10^7 total viable ADRCs. The number of cells injected into each damaged disc ranged from 3.51 x 10^7- 2.91 x 10^6 ADRCs per disc.

MRI assessments were based on coronal T2 images reviewed by 5 independent raters. At both 6 and 12 months there was a significantly greater density in discs treated with Cells/HA compared with those receiving No Intervention (p=0.019), and only marginal differences between Cells/HA and HA only. There was also a difference between HA only and No Intervention (p=0.0002). No statistical difference between the Cells/HA treatment and the Control was observed at either 6 or 12 months. While significant treatment differences in the binary MRI outcome were observed, there were no significant time differences.

Disc height analysis focused on treatment and time. Statistical analysis was considered to account the disc level assigned to each treatment and tested whether differences could be attributed to the disc level treated and to the treatment. Although there were significant differences seen between the interventions and the controls, no differences between the three interventions reached significance. There was a time effect, and within the same therapeutic intervention, there were also significant differences across the disc levels. Interestingly, disc levels receiving cells/HA did not differ with regard to level treated, while both other interventions demonstrated differences varying with respect to the anatomical level of the disc treated, i.e. in the HA only group, disc height was significantly greater in the L3-L4 disc compared with the same intervention at the L4-L5 disc (p<0.0043).

Histology assessment confirmed transplanted ADRCs were identifiable within the discs at both 6- and 12-month time points, suggesting that the long term cell durability in the disc may contribute to the matrix sustenance as seen in MRI, and the level-independent association of disc height maintenance.

Conclusions
This study provides evidence that ADRCs are efficacious and safe when injected into the intervertebral disc. Differences could be detected in the treated levels by MRI. At the 12-month follow-up, differences between the levels treated with Cells/HA and the Control level were not statistically different. Disc height improved with time for both the discs that received Cells/HA and HA only, although they remained different from controls. Intervertebral disc levels that received Cells/HA were independent of anatomy – each level treated with cells was statistically consistent and independent of the level treated, unlike the other two interventions which varied with the level of treatment. Based on the MRI evidence showing enhanced disc hydration, ADRC intervention may have broader clinical applications by potentially augmenting both treated and adjacent levels [1].

Finally, yield of ADRCs per gram of fat, and size and weight of the dogs were not correlated with outcome. From this study, therapeutic intervention with autologous ADRCs appears to offer a minimally-invasive, cell-based therapeutic with promising post-surgical value as an intervention with regenerative potential.

References
2. Ganey, Meisel, Hutton, Spine. 2003; 23:2609-20

Disclosures
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