

# From Mild to Severe Degeneration: Canine Disc Degeneration Treatment via Off-The-Shelf NP Cell Transplantation

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**INTRODUCTION:** Intervertebral disc (IVD) degeneration stands as a significant concern, affecting both quality of life and socioeconomic factors. The potential of cell therapy to address IVD degeneration has garnered attention, showing promise in curtailing degeneration.<sup>1</sup> However, debates persist over the extent of its effectiveness, particularly in more severe cases, where cell retention and nutritional support might pose challenges.<sup>2,3</sup> To address these uncertainties, our study aims to establish a canine induced disc degeneration model encompassing reliable induction of mild and severe degeneration grades, enabling our evaluation of the capacity of nucleus pulposus (NP) cell injection to mitigate different severities in degenerative cascades.

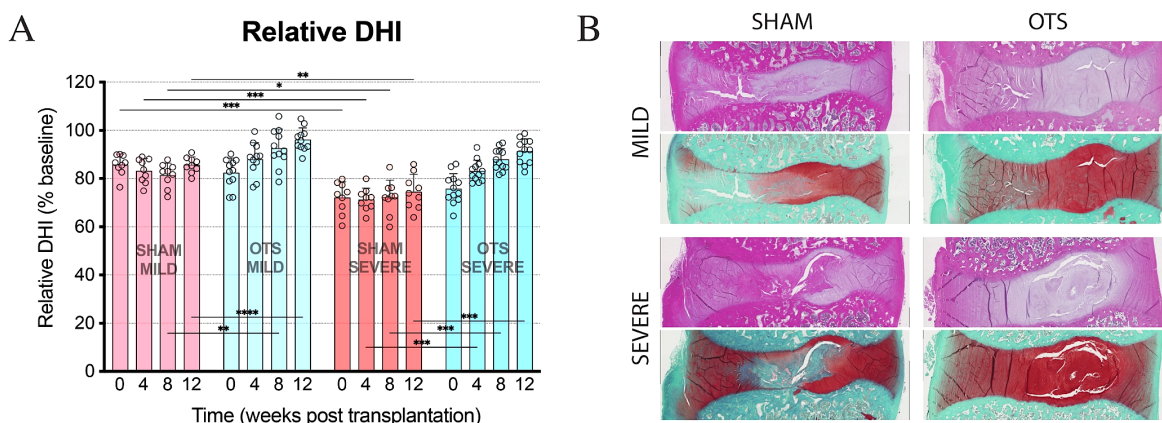
**METHODS:** Human NP cells were sourced and cultured from surgical waste tissue according to Sako et al.<sup>4</sup> (#17R173), optimizing their potency through tissue culture and FGF2 supplementation by enhancing Tie2 positivity<sup>5</sup>. Off-the-shelf NP cell suspensions (OTS) were cryopreserved for later injection.<sup>6</sup> Fourteen female chondrodystrophic beagles were randomly assigned: 7 for mild and 7 for severe degeneration induction in disc L3/4-L5/6 (L2/3 and L6/7 functioned as health reference). (#223014) Within each group, 3 dogs received sham saline injections, while 4 dogs received OTS-injections in the damaged discs. Under full sedation and fluoroscopy, mild degeneration involved aspirating 25-35 mg NP tissue using an 18G, ø1.2 mm, 70mm needle and 10mL syringe (≤3 attempts).<sup>7</sup> Severe degeneration employed a ø1.45 mm surgical punch, repeated 10-20 times aimed for a total of 40-50 mg tissue removal. After 4 weeks, 100 µL of saline or 1x10<sup>6</sup> cell OTS were injected. Canines underwent a 3-month observation period, evaluating disc height index, T2WI and T2\* MRI (3T, Phillips) monthly. Body weight and Glasgow composite scale were tracked biweekly. After study completion, canines were humanely euthanized, and histological examination of experimental discs was performed using the ORS spine histological grading in a blinded manner.<sup>8</sup> Temporal data were analyzed with 2-way ANOVA, non-temporal data with 1-way ANOVA via GraphPad Prism. A P-value below 0.05 is considered statistically significant.

**RESULTS SECTION:** All procedures were performed without complications. Mild degeneration involved an average of 29.6 mg tissue aspiration, while severe presented 41.9 mg aspirated tissue. Discs in the mild group resulted in about 16% (±5.5) and severe 26% (±6.5) DHI loss at time of transplantations. For sham-treated groups, no improvement was recorded, while OTS-treated discs showed progressive DHI increase, with at 12 weeks post-transplantation mild and severe discs reporting 95.2% (±5.8) and 91.4% (±5.1) respectively. (Fig 1A) MRI-derived T2 intensities showed more variable outcomes, but showed a trend of enhanced improvement in cell-treated discs in the mild condition, but not for severe outcomes. Pain assessment showed overall slightly worse scores for sham-treated dogs. Finally, histological observations (Fig 1B) confirmed DHI observations; sham-treated discs revealed a trend of worsened disc degeneration in the severe-group compared to mild-group. For both mild and severe discs treated with OTS, a reduction in scores was observed resulting in an average final score of 11.0 and 10.4 respectively, resulting in a significant difference comparing the OTS versus sham in severe degeneration group.

**DISCUSSION:** Assessing cell therapy within an appropriate disc degeneration model is paramount. The chondrodystrophic dog serves as an optimal mimic of the human condition.<sup>9</sup> Our study demonstrated successful induction of mild (~15% DHI loss) and severe (~25% DHI loss) states using our specific surgical methods. Notably, cell therapy yielded comparable outcomes for DHI recovery and histology in both cases. Despite incomplete recovery, initial degeneration severity didn't affect final results, implying potential applicability of cell therapy in severe degeneration. An existing study limitation is the uncontrolled disc inflammation state. Hence, the impact of chronic inflammation on severely degenerated discs and their response to OTS treatment warrants further investigation. Further work is ongoing to assess differences in the discs inflammatory state and overall cell retention in survival through immunohistochemistry.

**SIGNIFICANCE:** This study holds clinical significance by addressing the potential of OTS in both mild and severe cases of IVD degeneration, offering insights and highlighting the potential of cell therapy even in more severe cases.

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**Figure 1** (A) Relative disc height index compared to healthy baseline levels of the four different groups overtime. (B) Representative histological outcomes showing hematoxylin/eosin and safranin-O/fast green stainings for each condition.

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