Effects of Organ Culture and Mesenchymal Stem Cell Delivery on the Cellular Composition of the Nucleus Pulposus

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Introduction

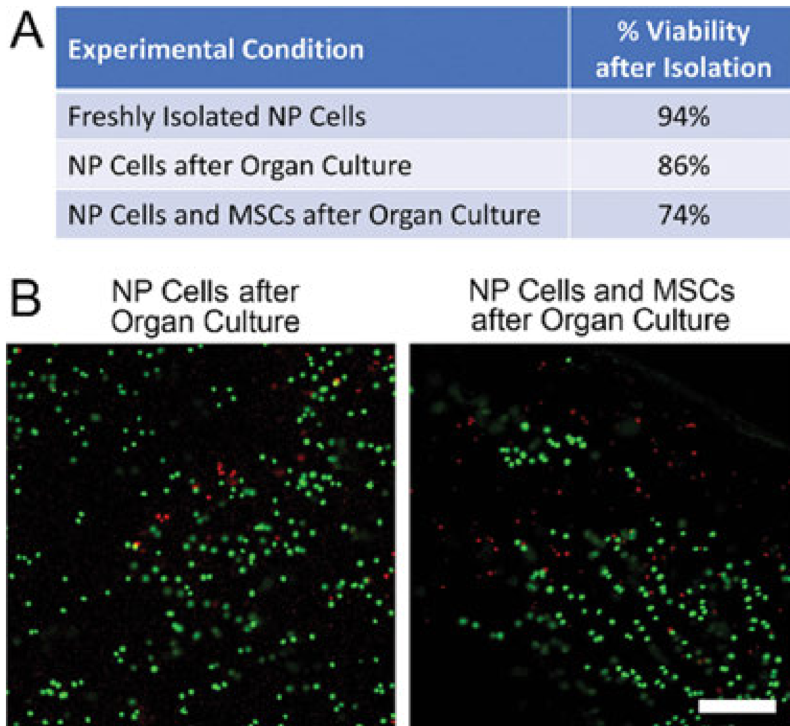
Intervertebral disc degeneration is a major cause of low back pain, the leading cause of disability worldwide.[1] Emerging cell-based therapies targeting the disc nucleus pulposus (NP), including those employing adult mesenchymal stem cells (MSCs), have shown promise in preclinical studies. However, consistent demonstration of efficacy and clinical translation is impeded by a poor understanding of the mechanisms of action. MSC-mediated mechanisms of action may include immunomodulation of endogenous cells, or direct reconstitution of native NP tissue.[2] Whole disc organ culture is a powerful preclinical tool for investigating such mechanisms under controlled conditions that closely recapitulate the *in situ* biochemical and biophysical microenvironments.[3,4] The objective of this study was to apply single cell RNA sequencing (scRNA-Seq) to investigate the effects of MSC delivery on the cellular composition of the NP using a whole disc organ culture model.

Methods

Lumbar spines were obtained postmortem from three male large frame goats, and discs were isolated and allocated to 3 experimental conditions: 1) NP cells freshly isolated from discs postmortem (i.e. no organ culture); 2) NP cells isolated after 7 days of whole disc organ culture; and 3) NP cells and MSCs isolated after 7 days of whole disc organ culture. For this final group, allogeneic adult goat bone marrow-derived MSCs (0.2×10^6 in 200μl saline) were injected into the NPs of discs on day 0. For all conditions, cells were isolated from the NP using a central 5mm biopsy punch followed by collagenase digestion. For organ culture, bony end plates were removed and discs were cultured with intact cartilaginous end plates in basal media (DMEM + 10% FBS) under limited swelling conditions [3]. For each condition, isolated cells from 5 discs were pooled, assessed for viability using trypan blue staining, and analyzed using scRNA-Seq. Libraries were generated using the Chromium controller (10X Genomics) and sequencing was performed using the Illumina HiSeq platform. Unsupervised clustering was conducted using Seurat and KEGG pathway analyses performed. Finally, one disc from each organ culture condition was assessed for cell viability using *in situ* live/dead staining.

Results

A total of 28,898 cells were sequenced (median 1284 genes/cell and 4904 UMIs/cell): 13,697 freshly isolated NP cells (94% viability), 7,637 NP cells after organ culture (86% viability), and 7,566 NP cells and MSCs after organ culture (74% viability). Lower viability for both organ culture conditions was confirmed by *in situ* live/dead staining (Figure 1). UMAP plots generated for each condition using identical analysis parameters identified ± unique cell clusters (Figure 2), with the percentage of cells present within each cluster differing between conditions. The number of cells in cluster 1 remained relatively consistent between conditions. In contrast, the percentage of cells in cluster 2 in both organ culture conditions was greatly diminished (< 7% of total cells). Cluster 5, largely absent in freshly isolated NP cells and NP cells alone after organ culture, comprised 28.15% of total cells for combined NP cells and MSCs after organ culture. In contrast, cluster 6, largely absent for freshly isolated NP cells, and combined NP cells and MSCs after organ culture, comprised 4.32% of total cells for NP cells alone after organ culture. Cluster-specific gene expression analyses performed for pooled cells across all 3 conditions (Figure 3) revealed that expression of NP-specific extracellular matrix (ECM) genes (ACAN, COL2A1 and COL6A3) was highest in cluster 2, and lowest in cluster 6. Expression of inflammatory/cell stress genes, including JUN, NFKBIA and PTGS2, was elevated in cluster ± vs other clusters. Pathway analysis also revealed down regulation of ECM-related and PI3K-Akt signaling pathways in both clusters 5 and ± compared to cluster 1, while there was upregulation of TNF-α signaling in cluster ± vs cluster 5.



Experimental Condition	% Viability after Isolation
Freshly Isolated NP Cells	94%
NP Cells after Organ Culture	86%
NP Cells and MSCs after Organ Culture	74%

Figure 1. (A) Percent viability of isolated cells from each condition prior to scRNA-Seq. **(B)** Representative in situ live (green) and dead (red) staining of cells for organ culture groups (scale = 100μm).

Figure 2. (A) UMAP plots showing the presence of six distinct cell clusters for each of the three experimental conditions. **(B)** Graph showing how the percentage of cells in each cluster varied between experimental conditions.

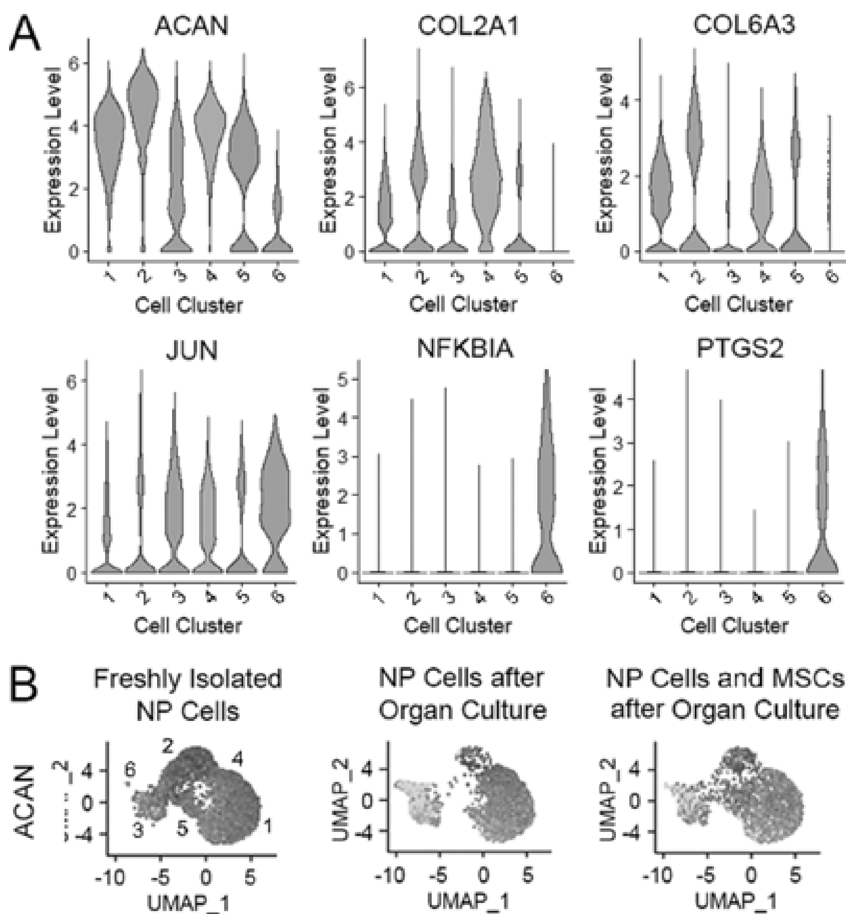


Figure 3. (A) Violin plots showing relative expression of ECM and inflammatory/cell stress genes in each cluster. **(B)** UMAP plots showing relative expression of ECM genes in each cluster.

Discussion

The goat is an established model for studying lumbar disc degeneration and cell-based therapies.[5] In this study we provide evidence of cell heterogeneity with the NPs of adult goat intervertebral discs, including distinct subpopulations characterized by unique gene expression profiles, a result that is consistent with recent scRNA-Seq studies of both bovine and human discs showing similar heterogeneity.[6,7] Organ culture models are used extensively as preclinical tools to study disc degeneration and therapeutic intervention.[3,4] Here we show that while organ culture did preserve some phenotypic properties of NP cells, there was an overall decrease in expression of key ECM genes after 7 days, potentially due to cell stress and/or de-differentiation. While discs were maintained under limited swelling conditions in this study, it is possible that bioreactors that apply physiological loading during culture[4] may be able to better maintain native cell characteristics. Delivery of MSCs resulted in suppression of pro-inflammatory signaling, supporting a potential immunomodulatory role for these cells.

Significance

Stem cell-based regeneration of the intervertebral disc is a promising treatment strategy for low back pain patients. In this study we provide insights into the cellular composition of the disc NP and potential mechanisms underlying MSC-based NP regeneration.

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