Interactions between Mesenchymal stem cells and Nucleus Pulposus cells: Implications for Intervertebral disc regeneration

INTRODUCTION:
Intervertebral disc (IVD) degeneration is a major cause of low back pain affecting westernized societies. Currently the mainstay of treatment for IVD degeneration is either symptomatic medical therapy or surgery, both of which have limited success. Cell based regenerative therapies offer an attractive solution as through implantation of appropriate cells it is possible to replace dysfunctional cells and repair/synthesize functional tissues.

Mesenchymal stem cells (MSCs) are a promising cell population for regeneration of the degenerate human IVD. Previous studies reported that direct cell-cell interaction between MSCs and nucleus pulposus (NP) cells stimulates the differentiation of MSCs towards NP like cells and enhances matrix gene expression within NP cells1. However, these studies did not consider the harsh microenvironmental “niche” of the degenerate IVD. Therefore the purpose of this study was to establish a model system in which MSCs: NP cells interactions could be studied and to ascertain the effect of hypoxia and reduced serum on this system.

METHODS:
Human bone marrow (BM) (n=3) and degenerate NP tissues (n=5) were obtained with consent during surgery in accordance with university and ethical committee policies and HTA legislation. MSCs expanded from BM were fluorescently labeled and co-cultured in monolayer with cell-cell contact with expanded degenerate NP cells at 50:50 ratio in either normoxia (20% O2) supplemented with 10% fetal calf serum (FCS) or hypoxia (2% O2) supplemented with 10% or 2% FCS for 7 days.

Following co-culture, cells were separated using fluorescence activated cell sorting. Quantitative real time PCR (QRT-PCR) was performed to assess expression of NP conventional marker genes (type II collagen (Col II), SOX-9, aggrecan (ACAN), Versican (VCAN)) and novel marker genes (PAX-1 and FOXF1) in MSCs and NP cells. All QRT-PCR data was normalized to the mean value of reference genes (MRPL19 and EIF2B1) using the 2^-ΔΔCt method. Statistical analysis was performed using the Mann-Whitney U-test and significance was defined as p <0.05

RESULTS:
Degenerate NP cells co-cultured with MSCs demonstrated a significant increase in expression of VCAN and PAX-1 in hypoxia. Serum reduction in hypoxia significantly increased expression of all conventional (Col II, SOX-9, ACAN, VCAN) and novel marker genes (PAX-1 and FOXF1) in co-cultured degenerate NP cells (Figure 1A).

MSCs co-cultured with degenerate NP cells showed significant up regulation of ACAN and VCAN in hypoxia. Serum reduction under hypoxia resulted in large and significant up regulation in expression of NP novel marker genes (PAX-1 and FOXF1) in co-cultured MSCs (Figure 1B). All other genes showed no significant change.

DISCUSSION:
The results demonstrated that IVD - like microenvironmental factors (low oxygen and reduced serum) influence the in vitro interaction between MSCs and degenerate NP cells. Close communication between MSCs and NP cells in reduced oxygen tension may release insufficient stimulatory signals to influence functionality of either cells type. Conversely hypoxia combined with reduced serum may favour the release of cellular components or growth factors from MSCs that reprogram the phenotype of degenerate NP cells for the self repair and from NP cells that initiate MSCs differentiation. However the precise relationship between metabolism and mechanism of communication regulating MSCs/NP cells behavior in an IVD-like microenvironment remains to be fully elucidated.

Figure 1: QRT-PCR analysis of NP conventional and novel marker genes in co-cultured NPCs (A) and MSCs (B).

SIGNIFICANCE:
This study demonstrates that MSCs and NP cells are still able to interact in an environment similar to that of the degenerate IVD “niche". Importantly it highlights the fact that model systems should mimic the in vivo situation as closely as possible to ensure that cells will function appropriately following implantation.

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REFERENCES:

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