Challenges for Cell Based Regeneration of the Degenerate Intervertebral Disc

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INTRODUCTION: Loss of intervertebral disc (IVD) matrix, predominantly in the central nucleus pulposus (NP), as a result of degeneration has been implicated as a major cause of low back pain. Current treatments are purely symptomatic and do not address the aberrant cell biology driving these matrix changes. However, cell based regenerative therapies offer an attractive solution to current interventions as through the implantation of appropriate cells it is possible to replace poorly functioning cells and repair and synthesize a functional tissue with similar features to the non-degenerate IVD. However, in order to develop a successful strategy a number of issues must be considered.

Choice of cells. The obvious choice would be the use of autologous NP cells, as with autologous chondrocyte implantation (ACI) for repair of cartilage defects. However, although clinical studies are underway in Europe (EuroDisc trial) with re-implantation of such cells, evidence from our laboratory and others would suggest that these cells are not ideal. For example, we have shown that cells from a degenerate disc show an altered phenotype (enhanced catabolism) and increased cell senescence which affects their expansion ability in culture (a prerequisite to generate sufficient numbers for re-implantation). Consequently attention has focussed on the use of mesenchymal stem cells (MSCs) because of their easy acquisition, rapid proliferation and differentiation potential.

MSC Differentiation: Data from our laboratory and others has shown that cells can be manipulated *in vitro* to differentiate to NP like cells by the addition of specific growth factors, manipulation of the culture environment and by seeding on suitable biomaterials (e.g. chitosan). Interestingly, recent data from our own co-culture studies would suggest that the interaction between MSCs and NP cells is sufficient to drive MSC differentiation to an appropriate phenotype and enhance matrix gene expression in degenerate NP cells. This, together with our data showing that cells injected into IVD tissue spontaneously differentiate into NP like cells implies that the IVD niche itself may be sufficient to direct cell differentiation and synthesis of appropriate matrix. A caveat to this however, is the environment of the degenerate IVD where oxygen tension and pH are low, nutrition is compromised, cells are exposed to mechanical load and there is a catabolic cytokine milieu, all of which may have detrimental effects on MSC differentiation. The effects of such factors on MSC differentiation to an NP phenotype are currently being investigated.

NP phenotype Although the NP cell shares many similarities with articular chondrocytes (AC) the matrix it produces and the environment in which it resides is distinctly different. Thus, for these therapies to be successful and appropriate matrix produced differentiated cells must have the correct NP phenotype. Using Affymetrix microarrays and qRT-PCR we have identified human gene markers that distinguish human NP cells from AC cells and have used these gene signatures to identify the differentiation of MSCs (derived from bone marrow (BM-MSCS) and Adipose tissue (ASCs)) towards an NP phenotype. Importantly we have demonstrated that our novel human phenotypic NP marker genes (e.g. PAX1 and FOXF1) and AC marker genes (IBSP and FBLN1) can be used to identify the *in vitro* differentiation of BM-MSCs and ASCs to an NP-like rather than an AC-like phenotype in biomaterials suitable for tissue regeneration of the human IVD. Furthermore our results indicate that ASCs may be a more appropriate cell type than BM-MSCs for repairing the human IVD.

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