Mimicking the Intervertebral Disc Microenvironment for Expansion of Nucleus Pulposus Progenitor Cells in a Context of Cell Therapy

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INTRODUCTION:Low back pain (LBP) is a global health concern that affects as many as 75–80% of people during their lifetime. Although the causes of LBP are multifactorial, increasing evidence implicates intervertebral disc (IVD) degeneration as a major contributor. In this respect, tissue-specific progenitors may play a crucial role for tissue regeneration, as these cells are perfectly adapted to their niche. Recently, progenitor cell population was described in the nucleus pulposus (NP) of the IVD. These cells, positive for Tie2 marker, have self-renewal capacity and in vitro multipotency potential. However, extremely low numbers of the NP progenitors limit the feasibility of cell therapy strategies. Here, we study the influence of the culture method and of the microenvironment on the human NP progenitors and their differentiation potential in vitro.

METHODS:Cells were obtained from human NP tissue from trauma patients undergoing spinal surgery. Briefly, after mild overnight digestion, the NP tissue cells were cultured in 2D (monolayer) or 3D (alginate beads) conditions with medium supplemented in ascorbic acid. After 2 weeks, cells from 2D or 3D culture were expanded on fibronectin-coating flasks with medium supplemented in FGF-2 to mimic the native microenvironment of NP cells. Subsequently, expanded NP cells were then characterized by cytometry (CD105, CD90, CD73, CD45, CD34 and Tie2) and tri-lineage differentiation, which was analyzed by qPCR and histology.

RESULTS:Cytometry analysis, after 2D- or 3D-expansion showed the presence of 0.1 % and 78.2 % of Tie2+ NP progenitors, respectively. Concerning the chondrogenic differentiation assay, the detection of glycosaminoglycans in the culture medium was drastically increased for 3D-expanded cells (11-fold) vs 2D-expanded cells. Moreover, the relative gene expression of collagen type 2 and aggrecan was also increased (600-fold and 2-fold, respectively). Regarding osteogenic differentiation assay, relative gene expression for osteopontin increased for 3D- (150-fold) vs 2D-expanded cells. However, no difference was observed between 2D and 3D expansion for the adipogenic differentiation assay.

DISCUSSION & CONCLUSIONS:The present study shows that 3D expansion of NP cells better preserves the progenitors cells population and increases the chondrogenic and osteogenic differentiation potential compared to 2D expansion. This project not only has a scientific impact by evaluating the role of native physiological niches on the functionality of NP progenitors but could also lead to an innovative clinical approach with cell therapy for IVD regeneration and repair.

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