Intervertebral Disc Regeneration Using Human Umbilical Cord Blood Stem Cell Therapy

Mick J. Perez-Cruet MD, MS; Jared Brougham; Rasul Chaudhry PhD; Naimisha Reddy Beeravolu MS
Oakland University William Beaumont School of Medicine, Oakland University Department of Biological Sciences

Introduction

Degenerative Disc Disease (DDD) is a major cause of chronic back pain. There is currently no biological cure for this debilitating disease. In DDD, the intervertebral disc (IVD) comprised of a fibrous outer ring (annulus fibrosus) and inner hydrated gel matrix (nucleus pulposus), becomes dry, fibrotic, and cracked as it degenerates. Human Umbilical Cord Blood (HCB) stem cell mediated therapy has the potential to restore the biological integrity of degenerated IVD (Fig 1). The effectiveness of HCB mesenchymal stem cells or chondroprogenitors for IVD regeneration has not been tested extensively.

Methods

An in-vivo rabbit model (n=20) of disc degeneration in the lumbar spine was created by flouroscopic guided needle puncture of the annulus fibrosis at the L2-3 and L4-5 level. The intervening L3-4 level acted as a control. At two weeks post puncture either the L2-3 or L4-5 laboratory created degenerated disc was subject to implantation of 1 million in 20ul i) undifferentiated human cord blood (HCB) mesenchymal stem cells or ii) differentiated HCB chondroprogenitor cells. Magnetic resonance imaging (MRI) was performed pre-operative, after inducing disc degeneration and 6 weeks post-implantation of stem cells. At 8 weeks post-implantation, rabbits were euthanized and control and experimental discs analyzed with haematoxylin and eosin (H & E) staining and immunohistochemical analysis (Fig 2). Biochemical analysis determined proteoglycan and glycosaminoglycan (GAG) content, and expression of specific markers to identify implanted cell viability (Fig 3).

Fig. 1.

Isolation of perinatal MSCs from human umbilical cord

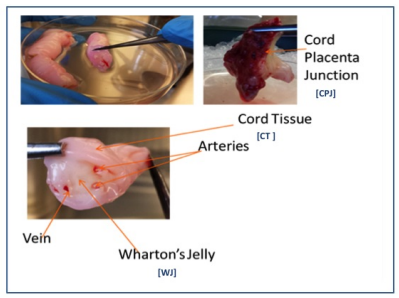


Fig. 2.

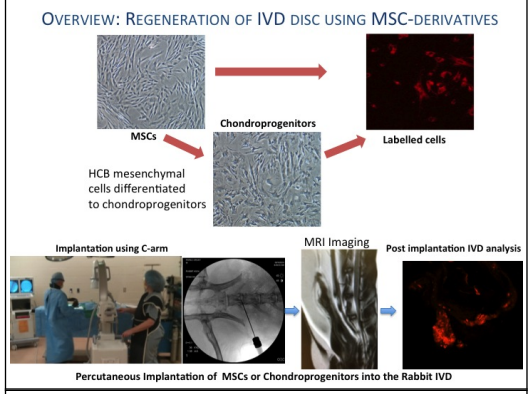


Fig. 3. Post-transplantation analysis of water content in IVD

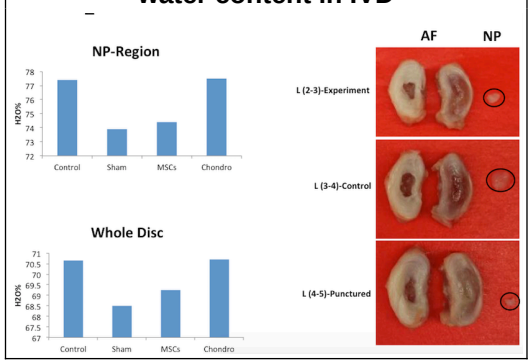
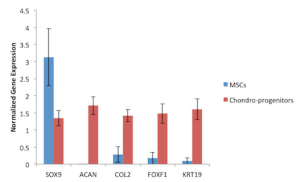


Fig. 4. Polimerize chain reaction expression of human markers in the rabbit IVD implanted with HCB.



- Upon transplantation of chondro-progenitors and MSCs into the degenerated disc in NP region-
  - Decreased levels of SOX9 represents in chondro-progenitors indicate more differentiation
  - The expression levels of the markers COL2, ACAN,FOXF1 and Keratin19 in MSCs were lower compared to chondro-progenitors
- This indicates that the chondro-progenitors are better source for the NP regeneration

Fig. 5. Periodic acid-Schiff and alcian blue staining for GAG in transpanted and control IVDs.

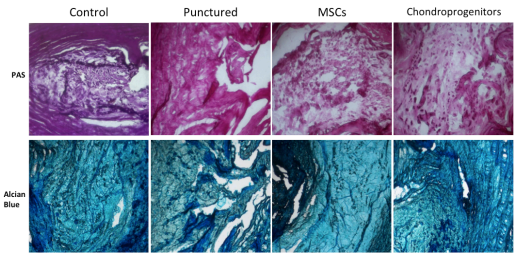
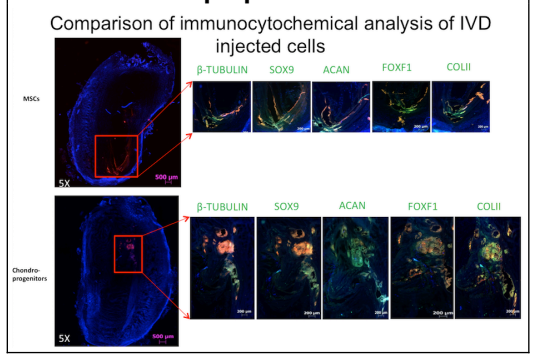


Fig. 6. Immunochemical analysis of nucleus pulposus markers.



Results

Successful extraction and culture of mesenchymal stem cells from the umbilical cord was confirmed by specific surface antigen markers (CD90, 73, 29, 105, and 44, Fig 4). The cells that were differentiated into chondroprenitor cells were found capable of producing GAG by alcian blue staining (Fig 5). MRI imaging demonstrated degeneration in punctured discs vaildating our model. H & E staining showed significantly more viable cell and extracellular matrix density in chondroprogenitor cell implanted degenerated disc. This was further shown with immuno-histochemical analysis of collagen type two content, implanted cell viability and disc rehydration (Fig 6).

Conclusions

i) HCB derived mesenchymal stem cells and chondroprogenitor cells appear to be effective in disc regeneration.

ii) Differentiated chondroprogenitor cells appear better suited for restoration of cellular and extracellular matrix within the nucleus pulposus.

iii) Percutaneous disc puncture is a safe reproducible method for inducing IVD degeneration.

References

1)Urban JP, Roberts S. Degeneration of the intervertebral disc. Arthritis Res Ther. 2003;5(3):120-30.

2)Sheikh H, Zakharian K, Perez De La Torre R, Facek C, Vasquez A, Chaudhry R, Svinarich D, Perez-Cruet MJ. In vivo intervertebral disc regeneration using stem cell-derived chondroprogenitors. J Neurosurg Spine. 2009;10(3):265-72.