Allogeneic Articular Chondrocyte Survived in the Degenerating Rabbit Intervertebral Disc

**In Vivo: Implications for Cell Therapy with Allogeneic Cells**


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**INTRODUCTION:**

Back pain is a common clinical problem that has an enormous socio-economic impact in today’s aging population. According to the United States Bone and Joint Decade report, estimated annual direct medical costs for all spine related conditions exceed $100 billion per year in the US. As an alternative to the surgical removal of the diseased disc, invasive fusion or artificial disc replacement, biological therapies that promote matrix repair and restore physiological function may be a future treatment option for disc degeneration.

With aging, progressive loss of the extracellular matrix of the intervertebral disc (IVD) occurs; cells undergo senescence or apoptosis, leading to disc degeneration. Transplanting cells which would repopulate the IVD and provide growth signals to resident cells by paracrine effects may be effective in reversing moderate to severe disc degeneration and reducing back pain. Since the disc tissue is relatively immune-privileged, allogeneic articular chondrocytes may be used instead of autologous articular chondrocytes thus avoiding an additional surgery on the same patient. An advantage of transplanting allogeneic articular chondrocytes into the IVD is that they have a chondrogenic phenotype which is similar to that of the IVD cells.

This study investigated the use of infrared, fluorescent and enzymatic methods for tracking allogeneic articular chondrocytes injected into degenerating rabbit IVDs.

**METHODS:**

After approval from the Institutional Animal Care and Use Committee was obtained, articular cartilage was harvested from the knees of euthanized young rabbits via sequential enzymatic digestion and cultured in vitro. To track cells using fluorescence microscopy and enzymatic methods, chondrocytes were transduced with recombinant adenovirus expressing red fluorescent protein (RFP) and β-galactosidase (β-gal) (Ad-RFP-β-gal). To track the cells after injection into the IVDs, the cells were labeled with Cellvue infrared dye (LI-COR Biosciences) according to the manufacturer’s instructions.

New Zealand White rabbit surgeries (n=5) were performed using a retroperitoneal approach to the lumbar spine. Three lumbar IVDs were injured by puncture with an 18G needle as previously described. Four weeks later, eight µl of transplanted/labeled chondrocytes was injected into injured IVDs at a concentration of 1X10^7 cells/ml. At two weeks (n=2) and eight weeks (n=3) post injection, the rabbits were sacrificed and the lumbar spines were harvested. Specimens were imaged with a LI-COR infrared scanner to detect location and distribution of Cellvue labeled cells. The IVDs were then sectioned and a β-gal assay was performed to detect functional β-gal activity. Specimens were also imaged with confocal microscopy to detect RFP expression.

**RESULTS:**

Articular chondrocytes tolerated both adenoviral transfection and infrared labeling without significant cytotoxicity. Using the LI-COR infrared imaging system, infrared dye-labeled cells are detected in the 800 nm channel and represented in green. The lumbar vertebral bodies are detected in the 700 nm channel and represented in red. When the infrared dye-labeled cells and vertebral bodies co-localize, the overlapped signals are represented in yellow (panels A and B). Subsequently, the individual IVDs were further separated at the growth plates to obtain intact IVDs with adjacent endplates. They were placed on the LI-COR scanner to obtain a transverse view of the each disc (panels A’ and B’). Arrows point to areas of intense infrared dye-labeling, which may represent the needle entry point. We have further shown that at 2 weeks or 8 weeks post-transplantation, the discs were stained for β-gal activity (data not shown). The injected cells were stained blue demonstrating production of the reporter protein. The transplanted cells were injected into the nucleus pulposus, and spread to the annulus likely through fissures induced by the needle injury. At 8 weeks post injection, the blue areas were smaller and less intense compared with 2 weeks post injection, likely due to decreased β-gal activity over time. In addition, at 2 or 8 weeks post-chondrocyte transplantation, respectively, transplanted cells in the NP tissues contain RFP by fluorescent microscopy (data not shown). Similar to the β-gal activity, RFP fluorescence intensity decreased at 8 weeks post-transplantation compared with the 2-week time point, suggesting decreased transgene expression. This is not surprising, because the adenoviral expression system is usually transient; the viral genes are not incorporated into the host genome. Cells may also differentiate and shut down over-expressing promoters; or cells may replicate and diffuse the recombinant adenosivirus genomes.

**DISCUSSION:**

These studies have demonstrated that transplanted cells labeled with infrared dye survived in the rabbit discs for up to 8 weeks. Transplanted cell presence and viability were further confirmed by transducing the chondrocytes with recombinant adenovirus Ad-RFP-β-gal. These studies confirmed that RFP and β-gal activity were still present in the cells injected into the discs, further suggesting that the cells may have remained viable. This study suggests that allogeneic cells may achieve long term survival in the relatively immune-privileged environment in the IVDs, thus provide a valuable source for cell therapy.

**SIGNIFICANCE:**

Three cell tracking methods have been utilized to demonstrate that cells injected into the discs remain viable in the tissue. These studies will help lay the groundwork for using cell therapy to treat disc degenerative diseases.

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**REFERENCES:**