# IN VITRO AND IN VIVO TRANSPLANTATION OF GENETICALLY-MODIFIED CHONDROCYTES TO CARTILAGE: A FEASIBILITY STUDY

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**RELEVANCE TO THE MUSCULOSKELETAL CONDITION:** Current therapies for osteoarthritis (OA) are only effective for alleviating symptoms. Cartilage transplantation has the potential to modify the OA disease process.

**INTRODUCTION:** In recent years, an improved understanding of normal cartilage biology and of the mechanisms that contribute to cartilage degeneration has encouraged exploration of methods for modifying the OA disease process. One potential approach is to genetically manipulate chondrocytes *in vivo* to enhance their production of extracellular matrix molecules and thereby replenish cartilage degraded by OA. However, it has become apparent that the ability of native chondrocytes to proliferate and elaborate substantial new matrix *in vivo* is limited. For example, when cartilage is injured, chondrocytes will proliferate and increase production of matrix molecules locally but will fail to bridge a defect or laceration. This suggests that it may be necessary to first provide a new source of chondrocytes to areas of substantial cartilage injury or loss prior to or in conjunction with chondrocyte manipulations that may increase matrix synthesis

The purpose of this study was to examine the feasibility of transplanting chondrocytes onto the cartilage surface both in organ culture and in vivo. In addition, we explored the possibility of genetically modifying endogenous and transplanted chondrocytes and we assessed the ability of these cells to continually express a gene product.

**METHODS:** Cartilage and chondrocytes from murine, canine and bovine knee joints were utilized. All experiments with animals were approved by the Toronto Hospital Animal Care Committee.

Chondrocytes were labeled with either of two fluorescent dyes, the red fluorescent membrane dye, Fm DiI (Molecular Probes) or the green fluorescent intracellular dye, Cell Tracker CMFDA (Molecular Probes). This allowed us to transplant two populations of cells at different times and follow both populations using confocal microscopy. In other experiments, chondrocytes were infected with the adenoviral vector AdlacZ that codes for β-galactosidase (J. Leiden, U. of Chicago). Labeled chondrocytes were transplanted to cartilage explants *in vitro* or injected *in vivo* into the animal knee joints.

**RESULTS:**

***In vivo binding of canine and murine chondrocytes to cartilage:***

Rat chondrocytes labeled with Fm DiI and injected into rat knee joints gave rise to an uneven distribution of labeled chondrocytes on both the femoral and tibial cartilage surfaces four days after injection. Rat cartilage directly labeled with Fm DiI *in vitro* was used to determine the density of autologous chondrocytes. An average of 550 chondrocytes/mm$^2$ of cartilage was noted; this is approximately 2.5 times the maximum density seen with *in vivo* transplants. With direct labeling the staining is uniform over the entire surface of the cartilage.

Similar experiments with Fm DiI labeled canine chondrocytes resulted in repopulation of the canine cartilage surface. Three injections into the joint space of the knees over a 27 day period resulted in extensive pockets of chondrocytes bound to cartilage by day 35. Chondrocytes were not distributed uniformly but rather in clusters of cells over the cartilaginous surface.

***In vitro transplant of bovine and canine chondrocytes to cartilage explants:***

The distribution of transplanted canine and bovine chondrocytes to cartilage explants was patchy over the surface of the femoral and tibial cartilage. Canine chondrocytes labeled with CMFDA dye, and transplanted to cartilage explants 10 days prior to the transplant of chondrocytes labeled with Fm DiI, were located as much as 1.6 µm below the Fm DiI-labeled cells as noted by confocal microscopy. This suggests that the transplanted chondrocytes were active in producing extracellular matrix within the cartilage.

***Transplantation of canine chondrocytes before infection with AdlacZ:***

Canine chondrocytes were infected with AdlacZ two days after transplant to organ culture to determine if the transplanted chondrocytes survived and the β-galactosidase gene introduced continued to be expressed. Chondrocytes infected two days after transplant continued to express β-galactosidase up to 60 days after transplant. When canine chondrocytes were transplanted to cartilage, successful infection occurred 1, 2, 5 and 8 weeks later, suggesting that chondrocytes remained accessible to virus particles. Furthermore, chondrocytes infected fifty-eight days after transplant continued to express β-galactosidase 73 days post transplant, the longest interval tested.

Direct infection of cartilage that had not previously undergone chondrocyte transplantation with AdlacZ resulted in very few blue foci compared to instances where the cartilage was resurfaced.

**DISCUSSION:** We have shown that transplanted chondrocytes continue to express the gene product β-galactosidase after infection with the adenoviral vector AdlacZ. Chondrocytes infected with AdlacZ two days after transplantation to organ culture continued to express β-galactosidase up to 60 days after transplant. Previous studies showed that transplanted chondrocytes adhered to human cartilage in organ culture [1,2] or to focal articular defects *in vivo* in rabbits [3] and continued to express transfected genes out to 10 and 28 days tested[2,3].

In addition, this study has demonstrated that transplanted chondrocytes can be infected efficiently well after transplant. Chondrocytes were successfully infected as late as 58 days after transplant. Since transplanted chondrocytes retain the ability to be infected by adenoviral vectors, repeat infections may restore expression if gene expression wanes over time. This may be an alternative to repeated injections of chondrocytes into the joint space.

Direct infection of endogenous chondrocytes gives many fewer infected cells. This suggests that transplanted cells are more easily infected than endogenous chondrocytes, possibly due to a different profile of cell surface viral receptors or as a result of steric factors that exclude viral particles from endogenous chondrocytes. Consequently, in order to repair and rebuild cartilage using transduced chondrocytes it will be necessary to resurface cartilage either with infected chondrocytes or with unmodified chondrocytes that will subsequently be infected. Confocal microscopy showed that canine chondrocytes injected into joints 10 days after an initial chondrocyte transplantation were located as much as 1.6 µm above the initially transplanted chondrocytes suggesting ongoing synthesis of cartilage matrix.

Our results to date raise the possibility of repairing cartilage by resurfacing damaged cartilage with transplanted chondrocytes. Furthermore, genetically modified chondrocytes, expressing anabolic growth factors such as TGF-β or IGF-1, may enhance the extracellular matrix reparative potential of transplanted chondrocytes.

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