ENGINEERING CARTILAGE FROM EXTRAMEDULLARY ADIPOSE TISSUE - A NOVEL SOURCE OF MESENCHYMAL STEM CELLS

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Introduction
Mesenchymal stem cells are used to engineer cartilage. Currently, these are procured from periosteum and bone marrow. The procurement of stem cells from these sources is highly invasive, tedious and gives a very low yield of cells. 1,2

Objectives
To investigate extramedullary adipose tissue as a source of mesenchymal stem cells and to show if fat could be transformed to cartilage.

Methods
Tissue for transplantation was harvested from a single New Zealand White rabbit. This allogeneous means of procurement is unique when compared to other implantation studies that use autogenous means of transplant. This was then purified and cultured using a process unique to our laboratories. Specifically this process involves the use of the lipid bearing components of adipose tissue as opposed to the stromal components used by other authors 3,4. In addition we have also perfected a means of culture which obviates the need for growth factors and cytokines in the media. Cells were allowed to grow to confluence as a monolayer before being passaged again. The cultured cells were characterised using a simplified protocol. Currently, there is no universally accepted means of characterising the mesenchymal stem cell. 1,3,5,6,7. Our protocol therefore involved morphological as well as functional characteristics of the cell. The ability to transform into a cell line other than cartilage was considered a prerequisite. Next, a pilot study was conducted to prove that the cartilaginous repair tissue formed in this process was derived from the cells that were transplanted. This was achieved by transfection of a batch of cells before transplantation with the XGal gene. The second phase of implantation formed the main bulk of this experiment. This was aimed at assessing the repair tissue morphologically and functionally. Thirty-seven New Zealand White rabbits were used. Cultured cells derived from fat or periosteum were transplanted into defects created in the left medial femoral condyle. Similar defects in the contralateral knee, the controls, were left empty to heal by native mechanisms. In addition, a biomechanical control was created where a osteochondral disc of cartilage was removed and replaced. This is a unique control - most studies use intact cartilage as a control which is not a fair standard on which to base the function of repair tissue. 1,8,9. All animals were returned to their cages after the operation and were allowed free movement. In the third phase the defects were assessed grossly, microscopically and biomechanically. Animals were euthanised via carbon dioxide inhalation. The femora were harvested. In the morphological assessment arm, the rabbits were sacrificed at 2, 6, 12 and 24 weeks respectively. These were assessed grossly measuring the residual osteochondral defects and histologically using the Pineda score. 1,10. In the biomechanical arm, the rabbits were sacrificed at 2 and 24 weeks. The creep modulus was measured from indentation tests using an Instron machine, where cartilage stiffness is described by elastic modulus under ramp loading and 15s creep conditions. 11. Intact cartilage and freshly created defects were also tested to provide the theoretical limits of physiological tissue in this injury repair model.

Results
Cells were more readily obtained from adipose tissue than from bone marrow or periosteum. There was no apparent need for specialised growth media or growth factors to improve the yield of cells as the present method also provided a better yield. Cells from adipose tissue reached confluence and were ready for passage in 4 days as opposed to the 7 day norm seen previously with cartilage, periosteum and bone marrow. The cells were characteristic of mesenchymal stem cells. They had a fibroblastic morphology and stained positively for Vimentin. In addition, they did not stain with Oil-Red-O and therefore were not adipocytes. Successful in vitro lineage direction into alternate mesenchymal cell lines revealed these to be pluripotent stem cells. This was achieved through transfection using azacytidine stimulation. The resultant cell population showed inherent contractile rhythmicity and stained positive for actin and myosin. They were therefore identified as cardiomyocytes. Unprocessed fat controls and periosteum derived stem cells both failed to differentiate into these cell lines. Studies on the origins of the repair tissue showed that the transplanted cells did indeed transform as expected into cartilage. The repair tissue here performed well. Gross osteochondral defect reconstitution and histological grading was superior to perium and stem cell repair and repair by native mechanisms. There was a tendency for the morphological results to progressively improve to be optimal at twelve weeks. Biomechanically, the repair tissue approximated intact cartilage and was superior to repair by native mechanisms. This difference was significant (p<0.05). There was also a significant difference between the performance of the osteochondral autograft control and intact cartilage. These results indicate that the previous means of comparing repair tissue to intact cartilage may not be a valid comparison as the act of disrupting cartilage itself changes its biomechanical characteristics considerably.

Discussion
Fat is a viable source of mesenchymal stem cells. The present method of stem cell procurement from adipose tissue offers a more efficient and clinically acceptable alternative to previous methods. The use of an allogeneous source has not been previously published and may have strong future implications.

References

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