

Explore the Potential of Human Lipoaspirate Stem Cells for Future Clinical Treatment of Cartilage Loss

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INTRODUCTION

Human adipose-derived stem cells isolated from lipoaspirate represent an attractive cell source for cell-based therapy due to the easy method of isolation, high yield from harvest and minimum ethic issue of use. Cartilage repair and treatment for cartilage loss using cell-based approach need a high amount of functional cells. These involved millions of cells that cannot be obtained from a cartilage biopsy in non-weight bearing knee cartilage of the same patient. Thus, the more practical way of treating cartilage damage will be using chondrogenic progenitor cells 'trained' from autologous stem cells.

OBJECTIVES

The main objective of this project was to explore the chondrogenesis potential of human adipose-derived stem cells and investigate the feasibility of using these stem cells for cartilage regeneration. The specific objectives were:

- 1). To determine the specific cell isolation technique from human lipoaspirate.
- 2). To define the culture medium for the expansion of human adipose-derived stem cells.
- 3). To define the chondrogenic induction medium for human adipose-derived stem cells.
- 4). To define the appropriate scaffold material for cartilage regenerative using human adipose-derived stem cells.
- 5). To evaluate the *in vivo* development of tissue-engineered cartilage in athymic mice model.

MATERIALS AND METHODS

Human adipose-derived stem cells (HADSC) were isolated from lipoaspirate from consented donor. Lipoaspirate tissue was digested with 0.3% collagenase type I for 2 hours at 37°C. Red blood cells were removed with lysis buffer to isolate the stem cells. HADSC were then cultured in F12/DMEM (1:1) growth medium with either supplementation of 10% fetal bovine serum (FBS) or human serum. HADSC were cultured until passage 5 for various stem cell surface markers identification, quantitative stemness gene expression as well as adipogenic and osteogenic differentiation tests for 3 weeks. HADSC were then tested for its *in vitro* chondrogenesis in a formulated chondrogenic medium that contained 1% ITS premix, 50ng/ml IGF-1, 50ug/ml ascorbate-2-phosphate, 100 nM dexamethasone, 40ug/ml L-proline and 5ng/ml TGF- β_3 with comparison to normal growth medium. Chondrogenic induction was carried out with high density culture of 1.0×10^5 cells/cm² in culture flask for 3 weeks. Total RNA from

cultures at first, second and third week in chondrogenic medium were extracted with TRI-Reagent. The expression level of chondrogenic genes were measured by quantitative RT-PCR. The specificity of the reactions was verified by melting curve analysis and gel electrophoresis. Primers used in this study were designed using Primer3 software based on the published GeneBank database sequences. *In vivo* chondrogenesis was tested by composited of HADSC with human fibrin. The cell-fibrin constructs were then implanted subcutaneously at the dorsum of athymic mice for 3 weeks. Constructs were then removed, fixed and evaluated by histological staining, immunohistochemistry and total sGAG quantification.

RESULTS AND DISCUSSION

The immunophenotyping assay showed HADSCs were positive for stem cell surface markers of CD90, CD73, CD44, CD9 and HLA ABC but negative for endothelial marker (CD31), hematopoietic marker (CD34), leukocyte marker (CD45) and HLA-class II. Positive Alizarin Red and Von Kossa staining on osteogenic induced HADSC revealed osteogenic differentiation of the cells. Osteogenic genes (OSC, RUNX, and ALP) were significantly upregulated ($p < 0.05$) after 3 weeks induction. The positive staining of Oil Red O and upregulation of adipogenic genes (LPL, AP2, and PPAR) demonstrated the adipogenic differentiation of HADSC. Human serum promoted significant higher proliferation rate of HADSC compared to FBS. However, stemness gene expression on SOX2, REX1, NANOG and OCT4 were reduced with human serum supplementation. During *in vitro* chondrogenesis, cells aggregation was shown in chondrogenic medium either added with or without basic fibroblast growth factor (bFGF). This cell aggregation during chondrogenic induction demonstrated the early stage of cartilage formation. The expression of cartilage specific markers such as collagen type II, SOX9, ACP and COMP were detected in both chondrogenic medium and medium supplemented with bFGF. Although SOX 9, COMP and ACP were expressed higher in chondrogenic medium group, collagen type II expression level were the highest in medium supplemented with bFGF at 3 weeks. The H&E histological staining showed homogenous distribution of cells in cell-fibrin construct. This revealed the suitability of fibrin as biomaterial for cartilage regeneration. After 3 weeks *in vitro* chondrogenic induction, Alcian Blue staining showed neocartilage formation. With an addition of 3 weeks *in vivo* development of cell-fibrin constructs in the nude mice, the cartilage markers of ACP and LINK were expressed in the implanted constructs. Total sGAG production in the chondrogenic group was higher than control group. The Alcian Blue staining also showed higher cartilage matrix accumulation in the chondrogenic group compared to the control group. In addition, collagen type II immunostaining revealed higher quality of neocartilage tissue production in chondrogenic group. However, the mature feature of chondrocytes was not demonstrated in any of the implanted tissue. This result illustrated that the cartilage tissue formed has not reach the mature stage and continue chondro-induction is needed for fully functional cartilage generation.

In conclusion, these data indicated HADSC shared similar multipotential with other types of adult stem cells. The human serum has higher mitogenic effect for HADSC but was less superior the FBS to maintain the stemness properties of HADSC. The chondrogenic medium was able to increase the chondrogenic genes expression of HADSC in *in vitro* culture. The human fibrin

revealed the good biomaterial for neocartilage formation. Although the implanted constructs did not develop into fully functional cartilage, current data showed the feasibility of using HADSC as cell source for cartilage regeneration when composited with fibrin as natural scaffold.