Chondrogenic-Differentiated Adipose-Derived Mesenchymal Stem Cell Sheets Exhibit Chondroprotective Properties in a Rabbit Model of Degenerated Articular Cartilage

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INTRODUCTION: Osteoarthritis (OA) causes degeneration of articular cartilage, which lacks the ability to self-repair. Additionally, despite numerous studies, effective therapies to repair articular cartilage remain elusive. Treatment of OA with adipose-derived mesenchymal stem cells (ADSCs) has recently attracted much attention in the field of regenerative medicine, with several reports demonstrating ADSC mediated protection of articular cartilage from degeneration. Additionally, while these cells do not regenerate or repair damaged cartilage, they express elevated levels of chondrocyte markers when cultured in platelet-rich plasma (PRP) containing media. In the present study, we successfully induced differentiation of ADSCs into chondrocytes in PRP-containing medium. Further, we employed ascorbic acid to prepare sheets of ADSCs that were induced to differentiate into chondrocytes, which were subsequently evaluated for their therapeutic effect on OA.

METHODS: Japanese rabbits with anterior cruciate ligament (ACL) transected for 4 weeks were used as OA models, and were assigned to 4 groups, namely the ADSC, ADSC sheet, PRP-ADSC, and PRP-ADSC sheet groups, and cells and cell sheets from each group were injected into the joint weekly from 4 weeks after ACL transection, and were subsequently sacrificed at 6, 8, 10, and 12 weeks in each group. Evaluation of Macroscopic OA score, OARSI score, immunohistochemical staining for MMP-1, MMP-13, and ADAMTS-4, and DiI labeling for cell tracking were carried out. And for *in vitro* assessment, the mRNA levels of representative chondrocyte markers, type Ⅱ collagen, aggrecan, and Sox-9 were examined in the ADSCs, ADSC sheet, PRP-ADSCs, and PRP-ADSC sheet groups using quantitative reverse transcription PCR. All data were analyzed and statistical significance was set at $p < 0.05$.

RESULTS: In vitro evaluation revealed upregulation of chondrocyte markers in ADSCs that were cultured in the presence of PRP, along with maintenance of gene expression post sheet formation. The PRP-ADSC sheet group demonstrated the greatest inhibition of articular cartilage damage as assessed by *in vivo* gross and histological evaluations from early timepoint. Additionally, maximal reduction in expression of MMP-1, MMP-13, and ADAMTS-4 was observed in the PRP-ADSC sheet group.

DISCUSSION AND CONCLUSION:

ADSCs can be easily harvested with minimally invasion of subcutaneous tissue and cultured in large quantities. Additionally, they are known to possess the ability to differentiate into various cell types, including osteoblasts, adipose tissue, and chondrocytes. ADSCs cultured in PRP containing medium demonstrated an elevation in chondrocyte markers, thereby suggesting genetic differentiation into chondrocytes. Gene expression was retained on addition of ascorbic acid that was employed to form sheets, thus enabling the creation of cell sheets with chondrocyte specific gene expression patterns. Notably, these when injected into joints demonstrated stronger inhibition of articular cartilage degeneration than either regular ADSCs or ADSC sheets. However, practical application of the PRP-ADSCs is limited by their inability to grow well on joint surfaces. ADSCs may exhibit articular cartilage protection and inhibit articular cartilage degeneration via paracrine effects that are exerted by soluble anti-inflammatory factors. In the present study, the greatest suppression of MMP-1, MMP-3, and ADAMTS-4 expression, which are collagenases and aggrecanase was observed in the ADSC-PRP sheet group, suggesting that these factors may play a strong inhibitory role in articular cartilage degeneration.

In conclusion, chondrogenic cell sheets, in which ADSCs were induced to differentiate into chondrocytes by PRP and formed a sheet structure using ascorbic acid, may survive in the synovium for a long time, secreting liquid factors with protective effects on chondrocytes and the cartilage matrix, which may inhibit the progression of cartilage degeneration.