Bone Regeneration Using Autologous Adipose-Derived Stem Cell Spheroid Complex

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INTRODUCTION:

Bone regeneration is one of the big challenges in stem cell research because bone defects cause functional disability. Recovery of skeletal function remains important for orthopaedic surgery. Failure of the treatment for a fracture or bone defect can affect the patient's activities of daily living and recovery to social activities.

In the present study, we set up a culture method for creating a large osteogenic adipose-derived stem cell (ADSC) spheroid complex by measuring the expression of protein in a sequential series of culture media and studied its osteogenic potential.

METHODS:

The purpose of this study was to prepare an ADSC spheroid complex with better osteogenesis potential. The ADSC spheroids were cultured in ascorbic acid–containing medium followed by osteoblast differentiation medium and were fused to create a spheroid complex. Each culture period was determined by analyzing the expression of collagen and other factors.

After culturing ADSC spheroids for 24 hours in Dulbecco's Modified Eagle Medium (DMEM), the spheroids were cultured in ascorbic acid–containing medium for five days followed by osteoblast differentiation medium. One day after exchanging to osteoblast differentiation medium, spheroids were collected and cultured for four days to obtain a spheroid complex. Each culture period was determined by analyzing the expression of collagen type I, alkaline phosphatase and integrin α5 to maximize the activity of ADSC spheroids.

Then an in vivo study was carried out to confirm the osteogenic potential of the ADSC spheroid complex created by the established protocol (Fig. 1).

RESULTS:

The expression of collagen type I increased significantly in ascorbic acid-containing medium ($p<0.05$) compared with control medium on day five, suggesting that culturing spheroids in ascorbic acid increases collagen synthesis. RNA was extracted from ADSC spheroids after 1, 3, 5, and 7 days in each medium and RT-PCR was performed to measure integrin α5 expression. The expression was transiently high on the first day of osteoblast differentiation culture and then gradually decreased. Osteoblast differentiation medium enhanced cell adhesion in spheroids.

The ADSC spheroid complex was made according to the above protocol and grafted into the rabbits to check the survival of the spheroid complex and osteogenesis in vivo. Spheroid complexes were transplanted into the lateral femoral condyle with no apparent adverse effects. In H+E sections, osteogenesis began by two weeks in the spheroid complex and beta-tricalcium phosphate groups, but not in the control group. There was more apparent bone regeneration in the spheroid complex group than in the other groups after eight weeks.

(Fig 2) Two weeks after transplantation in the control (a), beta-tricalcium phosphate (b), and ADSC spheroid complex (c) groups, osteogenesis had begun in the spheroid complex and beta-tricalcium phosphate groups, but not in the control group.

Eight weeks after transplantation (d), (e), and (f), respectively, there was more apparent bone regeneration in the spheroid complex group than in the other groups.

DISCUSSION AND CONCLUSION:

Following *in vitro* and *in vivo* study confirmed the osteogenic potential of the ADSC spheroid complex created by the established protocol. The ADSC spheroid complex stimulated bone regeneration and will be applied to the treatment of large bone defects.