USE OF A TISSUE-ENGINEERED OSTEOCHONDRAL GRAFT IN THE TREATMENT OF ARTICULAR CARTILAGE DEFECTS

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Introduction
Spontaneous healing of cartilage lesions is fairly limited because this tissue has a poor capacity to regenerate itself. These lesions have been widely treated by drilling into the subchondral bone, but long-term results generally have been disappointing. Recently, autologous chondrocytes implantation (ACI) has been developed 1), however, this technique was limited by the inability of the newly formed cartilage to fuse with the underlying subchondral bone. Osteochondral transplantation, so-called mosaic plastic 2), was able to repair not only cartilage but also subchondral bone. However, the limitation of graft and the problem of donor sites were not solved. Thus, we investigated the ability of a tissue-engineered osteochondral graft to heal cartilage defects in rabbit knees

Materials and methods
Preparation of a complex of beta-TCP block and chondrocytes in gel
Allogenic chondrocytes were prepared by 0.8% pronase and 0.4% collagenase digestion, and cultured as a monolayer in Dulbecco's modified Eagle medium (DMEM) +10 % FCS for 3 weeks. After removal from culture dish, the cells were suspended at 1 x 10^6 per 100 ul of x 2 DME + 20% FCS. The same amount of 3% of type I atelo-collagen solution was added to the cell suspension, and one drop of the mixture (approximately 15ul) were spotted on the beta-tricalcium phosphate (beta-TCP) block (4mm diameter, 5mm height). A cylindrical beta-TCP block used in this study was highly pure with 75 vol% porosity, and synthesized by a mechanochanical method. 3) The bilayer complex was allowed to gel in the incubator at 37 degrees for 15 minutes, and continued to culture for 24 hours with DMEM + 10% FCS (Fig. 1).

Experimental model
New Zealand White rabbits, weighing 3.0-3.2 kg, were used in this study. A medial parapatellar incision was made in the left knee, and a 4.2 mm diameter hole, penetrating the subchondral bone to reach the marrow, was drilled in the intercondylar groove of the distal femur. A complex of beta-TCP and chondrocytes in gel was press-fitted in the defect. All of the rabbits were returned to cage activity. Six rabbits each were sacrificed at 4, 8, 12, and 30 weeks after grafting. Defects filled with beta-TCP block alone were made as controls. The distal part of the femur was removed and fixed with 4% paraformaldehyde in phosphate buffered saline. After decalcification in 5% EDTA for 2-3 weeks, serial histological sections, which included the patellar groove, were cut.

Results
Macroscopically, one to two out of six rabbits at each observing time showed partial exposure of beta-TCP in the defects. On the other hand, the defects without exposure of beta-TCP demonstrated similar appearance of the adjacent cartilage. Histologically, four weeks after surgery, tarrate resistant acid phosphatase (TRAP)-positive multinucleated giant cells were directly contact to beta-TCP and osteoblasts were also present in bone marrow. At 8 weeks, newly formed bone was connected to adjacent bone. From 12 to 30 weeks after surgery, most of beta-TCP was replaced by bone, and only a small amount of beta-TCP remained underlying cartilage. Complete replacement by bone was found in some areas. Most of cartilage matrix was strongly stained with Safranin O, indicating rich in glycosaminoglycans. In contrast, the superficial layer was not stained, and the cell morphology was distinctly different from the deep levels of the reparative cartilage (Fig. 2). None of the control defects (beta-TCP block alone) showed cartilage formation.

Discussion
Large cartilage defects can lead to the osteoarthritic degeneration of articular cartilage, which often is seen after osteochondral fracture. The pathological findings of osteoarthritis is not only cartilage loss, but also abnormalities of subchondral bone and other factors. Thus, treatment of cartilage alone is not enough to repair osteoarthritis. Osteochondral graft can repair restricted osteochondral lesions such as osteonecrosis, however, limitation of graft obtained and donor site problem are present. In order to solve these problems, we investigated the possibility of a bilayer complex of beta-TCP and chondrocytes in collagen gel as a substitute of osteochondral graft. The results showed that beta-TCP was rapidly replaced by bone. This new bone have originated from the mesenchymal cells in bone marrow, as the beta-TCP was acellar. Histological examination at 4 weeks demonstrated that cell-based resorption of beta-TCP by TRAP-positive giant cells and osteoblastic apposition of new bone directly on the surface of beta-TCP, suggesting that a similar phenomenon to normal bone metabolism could be occurred in the defects of bone levels. In cartilage layer, proteoglycan rich in matrix and a vertical arrangement of chondrocytes were observed overlying subchondral bone. These results obtained from a rabbit osteochondral defect model suggest that this bilayer complex constructed with highly purified beta-TCP block and chondrocytes in collagen gel may be used to repair not only osteochondral defects but also osteoarthritis.

REFERENCES:

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