Preparation of cells and an injectable bone substitute

Allogenic chondrocytes were prepared by 0.8% pronase and 0.4% collagenase digestion, and were cultured as a monolayer in DMEM +10 % FCS for 2-3 weeks. After removal from culture dish, the cells were suspended at 1 x 10^6 per 100 ul of x 2 DMEM + 20% FCS. The same amount of 6.5 % of type I atelo-collagen (Koken Co.) was added to the cells. Beta-TCP granules (Olympus Biomaterials Co.) with 75% porosity were mixed with 6.5% of collagen to prepare an injectable bone filler. Five micrograms of rhBMP-2 were added to the injectable complex just before implantation.

Experimental model

New Zealand White rabbits, weighing 3.1-3.3 kg, were used in this study. A medial parapatellar incision was made in the left knee, and a 4.5 mm diameter hole, 4 mm deep in the subchondral bone, was drilled in the intercondylar groove of the distal femur. At first, the defect was filled with an injectable TCP containing 5 ug of rhBMP-2 to the level of subchondral bone, and then 6.5% collagen gel with or without chondrocytes was implanted to cover the surface and to repair cartilage layer. All of the rabbits were returned to cage activity. Six rabbits each were sacrificed at 6, 12, and 24 weeks after grafting. The distal part of the femur was removed and fixed with 4% paraformaldehyde in phosphate buffered saline. After decalcification, serial histological sections were cut.

Results

Macroscopically, four out of thirty-six rabbits 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, 6 weeks after surgery, most of beta-TCP was replaced by bone, and only a small amount of beta-TCP remained underlying cartilage. 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. At 12 weeks, beta-TCP granules were completely resorbed and replaced by bone. At 24 weeks, hyaline-like cartilage was still dominant in cartilage layer in both cell-seeded and non cell-seeded groups. There was no histological difference between two groups in term of bone and cartilage regeneration.

Discussion

ACI was limited by the inability of the newly formed cartilage to fuse with underlying subchondral bone. In addition, complications of periosteal hypertrophy and leakage of cells were reported. Osteochondral graft can repair restricted osteochondral lesions. However, limitation of graft obtained and issue of donor site are problematic. In addition, it is difficult to fill the complicated shaped bone defects with TCP blocks. In order to solve these problems, we investigated the possibility of a new technique which allowed to repairing both bone and cartilage. The results showed that beta-TCP was rapidly replaced by bone. This phenomenon was promoted by adding BMP-2. Histological examination demonstrated that cell-based resorption of beta-TCP by TRAP-positive giant cells and osteoblastic apposition of new bone on the surface of beta-TCP, suggesting that a similar phenomenon to normal bone metabolism could be occurred in the defects of bone levels. Interestingly, cartilage regeneration was found without chondrocytes at 24 weeks. It seemed that the newly formed subchondral bone and cartilage have originated from the mesenchymal cells in bone marrow. These results suggest that this technique using an injectable beta-TCP and rhBMP-2 without chondrocytes does not require cell proliferation and may be used to repair human osteochondral defects.