Case Report

Transplant of Mesenchymal Stem Cells and Hydroxyapatite Ceramics to Treat Severe Osteochondral Damage After Septic Arthritis of the Knee

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ABSTRACT. We describe a 21-year-old man with a large osteochondral defect of the knee after septic arthritis, successfully treated by transplant of mesenchymal stem cells (MSC) from bone marrow and a new type of interconnected porous hydroxyapatite ceramic (IP-CHA). We confirmed successful cartilage-like tissue regeneration by a second arthroscopy. Biopsy of the repaired tissue revealed cartilage-like regeneration and bone formation. We were able to regenerate new bone and cartilage-like tissue in a one-stage operation, without sacrificing autologous bone or other tissue. This cultured MSC and IP-CHA hybrid material transplant represents a novel treatment for a severe osteochondral defect after septic arthritis. (J Rheumatol 2005;32:1615–8)

Key Indexing Terms:
MESENCHYMAL STEM CELL HYDROXYAPATITE CERAMICS
OSTEOCHONDRAL DEFECT SEPTIC ARTHRITIS TISSUE ENGINEERING

Causes of septic arthritis of the knee include intraarticular injection, open fracture of the joint, or hematogenous inoculation. If it is not diagnosed promptly and treated properly, it causes extensive osteochondral damage, resulting in severe dysfunction of the joint[1]. A large osteochondral defect after septic arthritis is a problematic lesion, because it is very difficult to treat due to poor blood supply and extensive scar tissue formation.

Recently, regenerative medicine using tissue-engineering techniques has shown some potential for the treatment for such lesions, which are very difficult to manage with conventional treatments[2,3]. Tissue engineering consists of 3 major elements: transplanted cells, a scaffold for cell proliferation, and growth factors. Mesenchymal stem cells (MSC) from bone marrow are a cell population that can differentiate into several mesodermal lineages, and they are considered to be the most clinically promising stem cells[4-6]. As a scaffold for bone and cartilage regeneration, a new type of calcium hydroxyapatite [$Ca_{12}(PO_4)_6(OH)_2$] ceramic with interconnected pores (IP-CHA) was produced recently[7]. This new type of CHA has a systemic arrangement of uniform spherical pores, and almost all pores are connected through interconnecting holes, enabling simultaneous achievement of high porosity and high initial mechanical strength [porosity, 75%; mean porous diameter, 150 μm; interconnective pore hole, 10–90 μm (mean 40 μm); ≥ 90% of pores are interconnective]. Because this IP-CHA has interconnected pores that allow cells to invade deeply into pores, it has higher biocompatibility, bioaffinity, and osteoconduction, compared to other commercially available CHA.

We describe a patient with a large osteochondral defect after septic arthritis of the knee, successfully treated by transplant of the expanded MSC and IP-CHA.

CASE REPORT

In July 2001, a 21-year-old male construction worker sustained an open dislocation fracture of his right knee when he was hit by an oncoming vehicle while riding a motorcycle. He was transferred to a local hospital where an emergency operation was performed. The operation included osteosynthesis of an open medial femoral condyle fracture, repair of the medial and lateral collateral ligament, and medial meniscal repair. Thirteen days after the operation, pus discharge appeared from his right knee joint and the serum level of C-reactive protein increased to 18.6 mg/dl. Methicillin-resistant *Staphylococcus aureus* grew in the culture of the pus discharge. He underwent curettage of the lesion and continuous irrigation of the joint twice at the hospital. His right knee joint was immobilized for a long time for the purpose of resting.

He presented in April 2002 with severe right knee pain and limited range of motion of the joint. The flexion angle of the joint was severely limited to 40°. Mild posterior and lateral laxity of right knee joint was observed, although the laxity could be concealed by the joint contracture. He complained of severe right knee pain especially while walking, and his daily activity was severely restricted due to pain and motion disturbance of the joint. Magnetic resonance imaging, 3-dimensional computed tomogra-

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phy (CT), and diagnostic arthroscopy revealed an extensive osteochondral defect on the medial femoral condyle (20 × 25 × 25 mm), causing an audible click during range of motion of the joint. We suspected that the most problematic lesion of the joint was the large osteochondral defect of the medial femoral condyle and joint contracture. He was given detailed information about the several treatment options including conventional bone grafting.

In November 2002, with approval from the ethical committee of our university, bone marrow aspirates (20 ml) were obtained from the left tibia under local anesthesia, and bone marrow cells were harvested and expanded in a monolayer culture system. The aspirates containing cells were suspended individually in culture media consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% patient's serum, gentamicin sulfate (50 µg/ml) and amphotericin B (10 µg/ml), seeded on sixteen 100 mm culture dishes in total, and incubated in humidified 5% CO_2-95% air atmosphere at 37°C. The medium remained unchanged for the first 7 days, then was changed at intervals of 2 or 3 days. At the end of primary culture, adherent colonies were detached by treatment with 0.25% trypsin and 0.02% EDTA, and rinsed with culture medium twice. Thereafter, we referred to these cells as MSC. After culturing for 24 days, the total cell number of MSC was 1.15×10^6.

An IP-CHA (Toshiba Ceramics Co., Ltd., Tokyo, Japan) had been made to order according to the osteochondral defect size measured by 3-dimensional CT scans. After it was fixed to the osteochondral defect of the patient's right medial femoral condyle with 2 bioabsorbable pins, the cultured MSC suspended in 10 ml saline solution were infiltrated into the IP-CHA using a syringe. Thus, the cultured MSC and IP-CHA hybrid was transplanted to the lesion (Figure 1). Flexion of the right knee joint was improved to 140° after the operation. Range of motion exercise was started 3 days after the operation and partial weight-bearing was allowed 3 weeks after the operation. The right knee pain and click sound disappeared soon after the operation. The flexion angle of the knee joint was restored to 120°, enabling the patient to resume daily activities.

The second arthroscopy, one year after the operation, revealed that the osteochondral defect was repaired, with smooth cartilage-like tissue and no bony defects, although cartilage-like tissue regeneration was not complete and a small area of bony exposure was observed (Figure 2). At the second arthroscopy, a needle biopsy (2.0 mm diameter) was performed. Biopsy tissue was taken from the center of the regenerated cartilage-like tissue. The tissue was fixed with 10% buffered formalin for one day. The specimen was decalcified with 10% formic acid, dehydrated in graded alcohols, and embedded in paraffin wax. Specimens were cut sagittally into pieces 5 µm thick and the centers of the sections were stained with safranin O/fast green stain.

The biopsy specimen consisted of fibrous tissue in the superficial layer and cartilage-like tissue in the middle to deep layer, in which the extracellular matrix was stained densely with safranin O, showing good integration to the subchondral bone. However, the areas densely stained by safranin O were patchy, indicating that large areas of the regenerated tissue seemed to be deficient in proteoglycans. There was no tidemark and the surface of the regenerated tissue was irregular. Because the IP-CHA of the regenerated tissue was resolved through the procedures of decalcification, we could not observe the IP-CHA in the biopsy specimen (Figure 3). When last seen in December 2004, the patient was very satisfied with the results, especially since he had been able to return to work.

DISCUSSION

Septic arthritis can cause extensive cartilage degeneration with large bone defects, resulting in severe dysfunction of the joint. It is a major problematic disease especially for younger patients. Articular cartilage has poor intrinsic healing capacity because of a lack of blood vessels and its isolation from systemic regulation. Especially if cartilage defects are accompanied by large bone defects, it is very difficult to achieve the normal articular structure. Recently, regenerative medicine in which healthy tissue is generated *ex vivo* with tissue-engineering techniques has been thought to have potential for the treatment of osteochondral defects. Tissue engineering consists of 3 elements: transplanted cells, a scaffold for cell proliferation, and signaling molecules. As a cell source for musculoskeletal regeneration, MSC are of great interest in the orthopedic field. MSC are undifferentiated cells isolated from adult tissue, which have the capacity to differentiate into mesodermal lineages, such as bone, cartilage, fat, muscle, or other tissues. MSC from bone marrow can be expanded in culture and differentiated into the desired lineage with the application of specific growth factors or bioactive molecules. MSC have several potential advantages for tissue regeneration: (1) They can be isolated from bone marrow by aspiration and expanded in the usual culture system. (2) Immuno-reactions from the host tissue can be avoided through the use of autologous cells. (3) The ethical problems concerning use of embryonic stem cells are avoided. (4) MSC can be easily transduced by several vectors. We believe that these MSC from bone marrow are the most clinically promising stem cells.

Several reports describe use of MSC and CHA transplants to repair the critical size of bone defects. In 1999, Bruder, *et al* expanded MSC isolated from a normal human source, loaded them onto a ceramic carrier, and implanted them into critical-size segmental defects in the femur of adult athymic rats[8]. They bonded the MSC to the hydroxyapatite by incubating the hydroxyapatite in MSC suspension for 2 hours, applying a brief vacuum to draw the cell suspension into the pores. They observed radiographically, histologically, and biomechanically better bone formation than in cell-free control ceramics. Similarly, Kon, *et al* reported successful bone regeneration after transplant of MSC and CHA using a sheep model[9]. In that study, MSC were loaded onto the hydroxyapatite by gentle rolling of hydroxyapatite cylinders with MSC suspension on the day before surgery. In 2002, Quarto, *et al* reported 3 patients with large bone defects in their long bones, who had undergone transplantation of osteoprogenitor cells from bone marrow and macroporous CHA. They observed excellent bone regeneration and functional recovery with this treatment, although they did not describe details of coculturing the MSC and hydroxyapatite[10]. Thus, CHA has been used as a scaffold for bone regeneration because it has high biocompatibility and good bioaffinity, and stimulates osteoconduction. However, one disadvantage of previously used commercial CHA was that the pores of implanted CHA were not totally filled with newly formed host bone, because they had a closed structure with few interpore connections. IP-CHA is a new type of CHA with spherical pores of uniform size interconnected by window-like holes (porosity, 75%; average pore size, 150 µm; average interpore connections, 40 µm). This IP-CHA

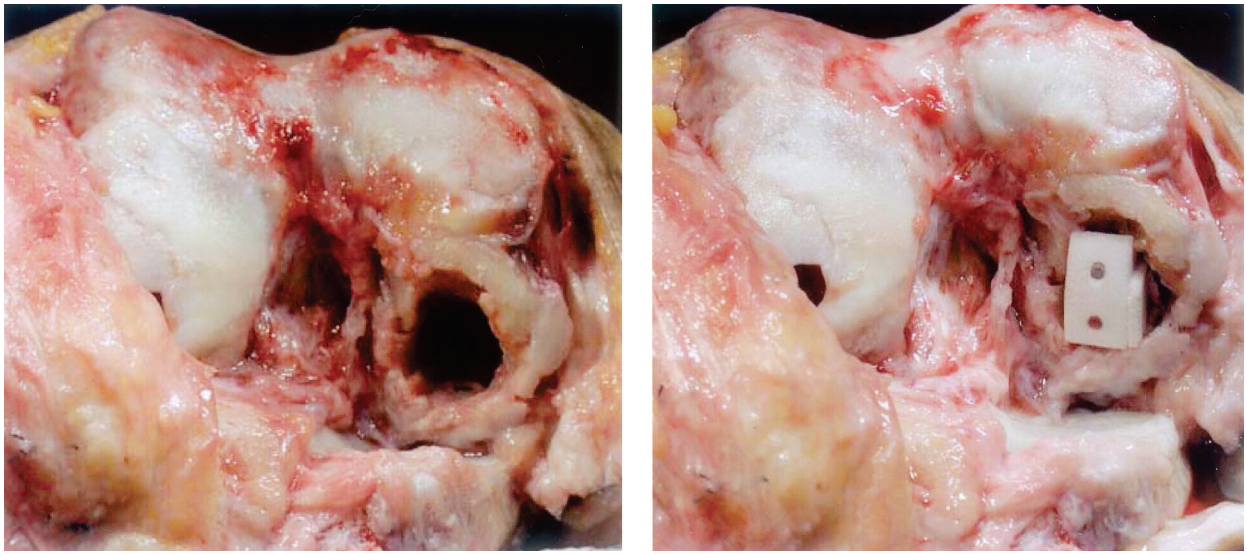

Figure 1. A. Large osteochondral defect (20 × 25 × 25 mm) on the medial femoral condyle. B. MSC (1.15×10^6) and IP-CHA are transplanted and fixed with 2 PLLA pins.

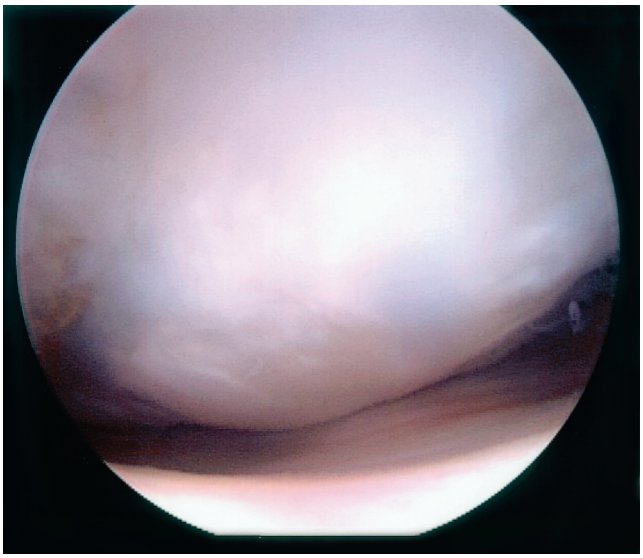

Figure 2. Followup arthroscopy shows the osteochondral defect is repaired with cartilage-like tissue.

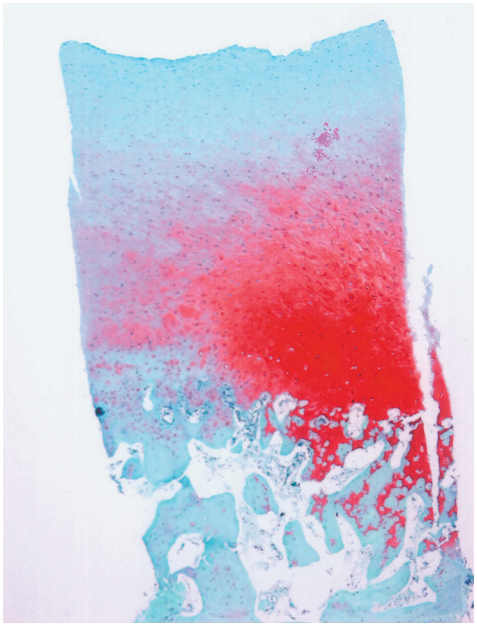

Figure 3. Biopsy of repaired tissue reveals cartilage-like tissue in the deeper zone and excellent bone regeneration (original magnification ×50, safranin O stain).

has high biocompatibility and bioaffinity and superior osteoconduction. It was reported that the interpore connection of IP-CHA appeared to enable superior osteoconduction by allowing cells and tissues to invade deeply into its pores. Moreover, if transplants of MSC and IP-CHA without coculture show sufficient osteogenic ability, it will provide several advantages for clinical use. Ito, *et al* devised bone defects in the tibial condyles of rats, and transplanted cylindrical IP-CHA that were hybridized with MSC without coculturing[11]. They reported that MSC/IP-CHA composite could enhance bone formation compared with the IP-CHA-only implant group. They also observed that injected MSC could survive in the IP-CHA and differentiate into osteoblast-like cells[11]. This MSC and IP-CHA transplantation without coculture has advantages of cost and ease compared with coculture, and thus may be suitable for clinical use.

We observed successful bone and cartilage-like tissue regeneration with cultured MSC and IP-CHA hybrid material. Although our result was confirmed by clinical findings

and arthroscopy, our report has some limitations. One is that followup periods of this patient were too short for evaluating longterm survival of the regenerative tissue, because as seen in the histology of the biopsy specimen, regenerated tissue was not true hyaline cartilage. There is a possibility that the regenerated tissue may deteriorate with time, causing joint dysfunction.

As noted, there have been reports in which good bone regeneration was confirmed after transplant of MSC and IP-CHA. However, this is the first study that has shown excellent bone and cartilage-like tissue regeneration after transplant of MSC and a new type of CHA for the treatment of large osteochondral defects of the knee. Although the mechanism of bone and cartilage regeneration and the cell source of the tissue was not clarified, it is possible that MSC could differentiate into chondrocytes and osteocytes given the appropriate microenvironmental stimulation. That we could regenerate new bone and cartilage-like tissue in a one-stage operation without sacrificing autologous bone or other tissues is clinically noteworthy. This cultured MSC and IP-CHA hybrid material transplant technique represents a novel treatment for patients with severe osteochondral defects of the knee joint.

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