Osteoarthritis and Cartilage (2002) **10,** 199–206
© 2002 OsteoArthritis Research Society International
doi:10.1053/joca.2001.0504, available online at http://www.idealibrary.com on IDEAL®
1063–4584/02/030199+08 $35.00/0

Osteoarthritis and Cartilage
Journal of the OsteoArthritis Research Society International

Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees

S. Wakitani*, K. Imoto†, T. Yamamoto†, M. Saito†, N. Murata† and M. Yoneda‡
**Department of Orthopaedic Surgery, Sinshu University School of Medicine, Japan*
†Department of Orthopaedic Surgery, Osaka-Minami National Hospital, Japan
‡Center for Sports medicine, Osaka Kosei-Nenkin Hospital, Japan

Summary

Objective: There is no widely accepted method to repair articular cartilage defects. Bone marrow mesenchymal cells have the potential to differentiate into bone, cartilage, fat and muscle. Bone marrow mesenchymal cell transplantation is easy to use clinically because cells can be easily obtained and can be multiplied without losing their capacity of differentiation. The objective of this study was to apply these cell transplantations to repair human articular cartilage defects in osteoarthritic knee joints.

Design: Twenty-four knees of 24 patients with knee osteoarthritis (OA) who underwent a high tibial osteotomy comprised the study group. Adherent cells in bone marrow aspirates were culture expanded, embedded in collagen gel, transplanted into the articular cartilage defect in the medial femoral condyle and covered with autologous periosteum at the time of 12 high tibial osteotomies. The other 12 subjects served as cell-free controls.

Results: In the cell-transplanted group, as early as 6.3 weeks after transplantation the defects were covered with white to pink soft tissue, in which metachromasia was partially observed. Forty-two weeks after transplantation, the defects were covered with white soft tissue, in which metachromasia was observed in almost all areas of the sampled tissue and hyaline cartilage-like tissue was partially observed. Although the clinical improvement was not significantly different, the arthroscopic and histological grading score was better in the cell-transplanted group than in the cell-free control group.

Conclusions: This procedure highlights the availability of autologous culture expanded bone marrow mesenchymal cell transplantation for the repair of articular cartilage defects in humans. © 2002 OsteoArthritis Research Society International

Key words: Bone marrow mesenchymal cell, Transplantation, Osteoarthritis, Cartilage defect, Regeneration.

The capacity of articular cartilage for repair is limited[1]. To repair articular cartilage defects, transplantations of various tissues or cells have been investigated both in animal and human models[1]. To repair articular cartilage defects in humans, mosaic plasty[2] (transplantation of multiple, small, autologous osteochondral grafts) and autologous cultured chondrocyte implantation[3] are effective. However, these are not applied to articular cartilage defects in osteoarthritis (OA) or rheumatoid arthritis (RA) because chondrocytes from patients suffering from OA or RA have totally different biological properties. For large articular cartilage defects, such as those of OA or RA, we have no repair method but can perform a total joint replacement for elderly patients with severely damaged joints.

It has been reported that the mesenchymal cells in bone marrow contain progenitor cells of some mesenchymal tissues, such as bone, cartilage, fat and muscle[4–6]. Bone marrow mesenchymal cells are thought to be useful for reconstructing injured tissues such as bone, cartilage, or cardiac muscle[4,7,8]. Such reconstructions have been studied in various animal models, but very few human studies have been made[9]. This procedure is easy to perform clinically because the autologous bone marrow mesenchymal cells are easy to obtain and can be culture expanded without losing their capacity for differentiation[10]. Here, we tried to use autologous human culture expanded bone marrow mesenchymal cells to repair large articular cartilage defects in the knee joints of patients with OA.

Received 26 February 2001; revision requested 9 July 2001; revision received 7 August 2001; accepted 26 September 2001.
This study was supported in part by a Grant from Japan Orthopaedics and Traumatology Foundation, Inc. No.114.
Address correspondence to: Dr Shigeyuki Wakitani, Department of Orthopaedic Surgery, Shinshu University School of Medicine, Asahi 3-1-1, Matsumoto 390-8621, Japan. Tel: 81-263-37-2659; Fax: 81-263-35-8844; E-mail: wakitani@hsp.md.shinshu-u.ac.jp

Patients and methods

PATIENTS

Twenty-four knees of 24 patients with medial unicompartmental OA who underwent a high tibial osteotomy were the objective of this study. Fifteen were female and 9 were male. The patients' average age was 63 (range 49–70). Their OA was diagnosed according to the criteria for the classification of OA of the knee prepared by Altman *et al.*[11]. Radiographic evaluations revealed that all the knees in this study had narrowing to complete obliteration of the medial compartment correlating with stage I to II Ahlback changes[12]. X-ray films of the knees were made both in weight-bearing and in non-weight-bearing conditions. OA of the lateral compartment or that of the patello-femoral joint was not observed or was small (the formation

of small osteophytes on the joint margins might be observed, but joint space narrowing was not).

Patients were randomly stratified into two groups. Twelve knees received autologous bone marrow mesenchymal cell transplantation and 12 knees served as cell-free controls. There were no differences in age and severity of the disease between these two groups.

Before and after surgery, all patients rated their pain (30 points), function (22 points), range of motion (18 points), muscle strength (10 points), flexion deformity (10 points), and instability (10 points) using the Hospital for Special Surgery knee-rating scale[13].

The procedure was in accordance with the ethical standards of our hospital committee on human experimentation. Patients were informed thoroughly and this surgery was only performed on those who wanted to receive this treatment.

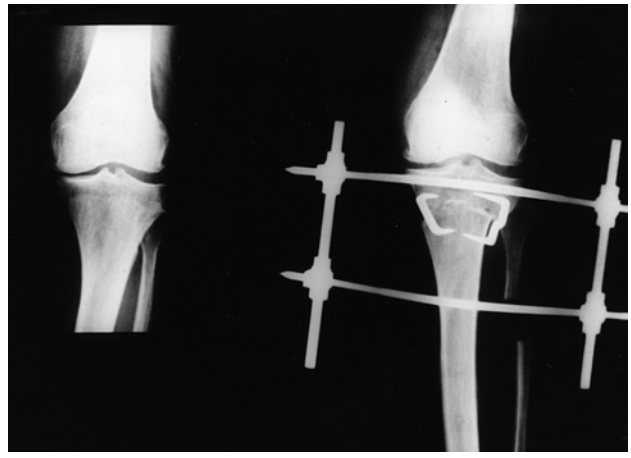

Fig. 1. A-P radiograms of the left knee in non-weight bearing condition. The left panel shows that before surgery. Narrowing of the medial joint space and formation of small osteophytes can be observed. The right panel shows that after surgery. High tibial osteotomy was performed using dome osteotomy, fixed with 2 Steinman's pins with Charnley's clamps and 2 staples.

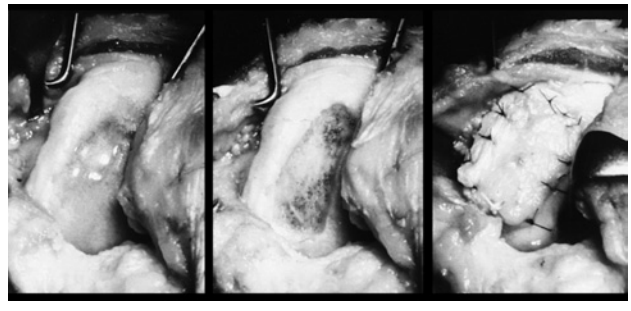

Fig. 2. Weight-bearing surface of the medial femoral condyle. The left panel shows that before surgery. Articular cartilage was lost, and subchondral bone was exposed and eburnated. The center panel shows that after abrasion of the eburnated bone. The right panel shows that after suturing the periosteum. A gel–cell composite was put on the abraded area, and the autologous periosteum was put on the cells the cambium layer facing down and sutured onto the remaining cartilage and/or bone.

BONE MARROW MESENCHYMAL CELL PREPARATION

Ten ml of heparinized bone marrow blood was aspirated from both sides of the iliac crest approximately 2 cm cranial from the antero-superior iliac spine. Heparinized bone marrow blood was mixed with a one-fifth volume of 6% (w/v) dextran (molecular weight 180,000–210,000; Nacalai Tesque Inc., Kyoto, Japan) in saline and left standing for 30 min at room temperature to eliminate erythrocytes. The remaining cells were washed twice with DMEM (Gibco BRL, Grand Island, NY) containing 10% FCS (Gibco BRL, Grand Island, NY) and antibiotics (100 unit/ml of penicillin, 0.1 mg/ml of streptomycin, 0.25 μg/ml of amphotericin B; Sigma Chemical Co. St Louis, MO). The mean number of nucleated cells was 1.0×10^7 cells. These cells were cultured in 4 dishes (100 mm in diameter) in DMEM supplemented with 15% FCS and antibiotics in a humidified atmosphere of 5% CO_2—air at 37°C. The medium was changed twice a week. After about 3 days, attached cells were recognized. At the time of medium change, non-adherent cells were removed with the medium. After approximately 10 days when the attached cells became subconfluent, they were detached with trypsin-EDTA solution (0.25% trypsin, 1 mM of EDTA-4Na; Gibco BRL, Grand Island, NY) and subcultured into 12 dishes (100 mm in diameter). After approximately 20 days, on the day before surgery the cells were collected (mean cell number was 1.3×10^7). They were embedded in 2 ml of 0.25% type I acid soluble collagen from porcine tendon (Cellmatrix type I-A, Nitta gelatin Inc., Osaka, Japan), put onto a collagen sheet (Koken Inc., Tokyo, Japan) and gelated. This gel–cell composite was cultured overnight in DMEM supplemented with 15% autologous serum and antibiotics.

SURGICAL PROCEDURE

High tibial osteotomy was performed using dome osteotomy, fixed with two Steinmann's pins with Charnley clamps and two staples (Stryker Howmedica Osteonics, Allendale, NJ). At first, a small part of the fibula was resected to facilitate the movement of distal tibia after tibial osteotomy. The first pin was inserted at the proximal end of the tibia parallel to the joint surface. The second pin was inserted in the middle of the tibial diaphysis at the planned correction angle (lateral side open). Then, the tibia was cut in a dome just proximal to the tibial tuberosity. The two inserted pins were set parallel (the alignment was corrected) and fixed with Charnley clamps. In the medial and lateral sides of the cut bone, one staple was inserted to fix the bone (Fig. 1).

CELL TRANSPLANTATION

The knee joint was opened by the parapatellar medial approach. The medial femoral condyle and medial tibial plateau were observed. In all cases, the articular cartilage on the medial femoral condyle was lost, and the subchondral bone was eburnated (Fig. 2). All knees had an Outerbridge stage IV[14] lesion on the tibial plateau and femoral condyle.

In 12 knees of the cell-transplanted group, 2 mm of the eburnated bone was abraded using a dental drill until slight bleeding was observed. To facilitate bleeding, multiple perforation was performed on the abraded area. The mean width of the abraded area was 14 mm×35 mm. The gel–cell composite was put on the abraded area with the collagen sheet covering the upper side. The autologous periosteum taken from the anterior surface of the proximal

tibia was put on the cells with the cambium layer facing them, and sutured onto the remaining cartilage and/or bone with non-absorbable nylon sutures (Fig. 2). To suture on bone, we made holes through the bone with 1.5 mm Kirshner wire, passed threads into them, and sutured the periosteum. Eight to 12 sutures were used to fix one periosteum.

All 12 knees of the cell-free control group received spongialization (exposure of cancellous bone), collagen gel-sheet implantations, and periosteal cover. Six received multiple perforations and the other six received abrasion of the eburnated subchondral bone with multiple perforation. Multiple perforation was performed on the bone to facilitate bleeding by a 1.2 mm Kirshner wire applied with an electric drill.

POST-OPERATIVE PROGRAM

Four days after surgery, continuous passive motion was applied. Three to 6 weeks after the surgery, partial weight bearing was started and by 8 weeks full weight bearing was permitted. Four to 10 (mean; 6.7) weeks after surgery, the pins were removed. At the time of this removal surgery, arthroscopy was performed (first look) with informed consent. The transplants were observed, and a small sample of repair tissue was obtained from macroscopically best repaired area for histology using biopsy needle. All patients received this surgery.

Twenty-eight to 95 (mean 42) weeks after the surgery when bone fusion was confirmed on the X-ray films, the staples were removed. At the time of the third surgery, arthroscopy was performed (second look) with informed consent. The transplants were observed, and a small sample of repair tissue was obtained from macroscopically best repaired area for histology. Nine of 12 cell-transplanted patients, and six of 12 cell-free patients received this surgery.

ASSESSMENT OF REPAIR TISSUE

The arthroscopic photography and histological samples of repair tissues from each patient were assessed independently by three of the authors (SW, KI, TY) who had no knowledge of the study group from which they had been obtained. Each sample was graded using an arthroscopic and histological grading scale, which was a modification of those described by Shino *et al*.[15] and Wakitani *et al*.[7]. The scale is composed of seven categories, five for arthroscopic and two for histological assessments, and assigns a score ranging from 20 to 0 points (Table I). The width of the reparative tissue was the relative width of the area covered with the reparative tissue compared to the original defect, which was graded from 3 (wider than 75%) to 0 (narrower that 25%). The thickness of the reparative tissue was the average thickness of the reparative tissue in the original defect compared to the adjacent normal cartilage, which was graded from 3 (thicker than 75%) to 0 (thinner than 25%). The surface regularity was the width of the smooth area of the reparative tissue compared with the original defect, which was graded from 3 (wider than 75%) to 0 (narrower than 25%). The stiffness of the reparative tissue was graded from 2 in which the repair was as stiff as normal articular cartilage, to 0 for reparative tissue that was easily depressed by a probe. The color of the reparative tissue was graded from 1 in which it was as white as normal cartilage, to 0 for other colors. The cell morphology was graded from 4 in which more than 80% of the reparative tissue was comparable to hyaline cartilage, whose cells showed round to polygonal shape, to 0 when cartilagenous tissues were absent. Matrix staining, or the degree of metachromatic staining with toluidine blue, was composed of two categories. The width was graded from 2 in which the area showing metachromasia was wider than two third of the whole section, to 0 in which the area was narrower than one third. The intensity was graded from 2 in which metachromasia was as good as normal hyaline cartilage, to 0 in which there was no metachromatic staining.

Table I
Arthroscopic and histological grading scale for reparative tissue

Arthroscopic assessment	
A. Width of reparative tissue: width of cover area with reparative tissue compared with the original defect.	
More than 75%	3
50–75%	2
25–50%	1
Less than 25%	0
B. Thickness of reparative tissue: average thickness of the reparative tissue compared with adjacent normal cartilage.	
More than 75%	3
50–75%	2
25–50%	1
Less than 25%	0
C. Width of surface regularity: width of smooth surface compared with the width of the original defect.	
More than 75%	3
50–75%	2
25–50%	1
Less than 25%	0
D. Stiffness of reparative tissue.	
As stiff as normal cartilage	2
Slightly reduced	1
Markedly reduced	0
E. Color of the reparative tissue:	
White	1
Not white	0
Histological assessment	
F. Cell morphology: cells showing a round to polygonal shape similar to hyaline cartilage.	
Mostly similar to hyaline cartilage (>80%)	4
3/5–4/5	3
2/5–3/5	2
1/5–2/5	1
Less than 20%	0
G. Matrix staining:	
Width of area showing metachromasia	
More than 2/3	2
1/3–2/3	1
Less than 1/3	0
Intensity of metachromasia	
Normal	2
Reduced	1
Non	0
Total maximum	20

STATISTICAL ANALYSIS

Statistical analysis of the clinical data was performed using the Wilcoxon signed rank test. Comparisons of the arthroscopic and histological grading scores between the cell-transplanted and cell-free groups were performed using the Mann–Whitney U-test. Statistical analyses of the

Table II
Clinical evaluation before and after surgery using the Hospital for Special Surgery knee-rating scale

	Mean (standard deviation)			
	Cell-transplanted		Cell-free	
	Before	After	Before	After
Total	65.0 (6.7)	81.3 (8.6)*	66.3 (10.5)	79.2 (8.7)*
Pain (30)	19.2 (2.9)	25.8 (3.6)*	18.8 (3.1)	24.1 (5.1)*
Function (22)	13.9 (2.6)	18.5 (3.0)*	13.8 (3.3)	16.5 (2.1)*
Range of motion (18)	15.8 (1.8)	14.1 (3.2)	14.5 (3.4)	14.9 (2.7)
Muscle strength (10)	6.0 (2.1)	8.5 (0.9)*	6.3 (2.1)	8.5 (0.9)*
Flexion deformity (10)	8.6 (1.0)	8.8 (1.0)	8.5 (0.9)	8.8 (1.0)
Instability (10)	7.5 (1.2)	8.5 (0.9)	7.8 (0.9)	7.9 (1.1)
Subtraction	4.2 (0.6)	2.7 (0.5)	3.8 (0.9)	2.9 (0.8)
Follow up periods (month)	14.3 (6.9)		17.5 (10.5)	

*Significantly improved (Wilcoxon signed rank tests).

improvements of the arthroscopic and histological grading scores were performed using the Wilcoxon signed rank test. Probability values less than 5% were considered significant. We used StatView (SAS Institute Inc., Cary, NC) to perform the statistical analysis.

Results

CLINICAL EVALUATIONS

The mean femoro-tibial angle before surgery in the weight-bearing condition was 184° and that in the non-weight-bearing condition was 181°. The mean femoro-tibial angle after surgery in the non-weight-bearing condition was 170°.

Clinical evaluations before and after surgeries were assessed using the Hospital for Special Surgery knee-rating scale. The mean follow-up period was 16 months. There was no difference in the follow-up period between the cell-transplanted and cell-free control groups (Table II). For the cell-transplanted group, the mean total score was 65.0 points before surgery, and 81.3 after surgery, which was significantly improved (P=0.0029). The scores of pain, function and muscle strength were significantly improved. For the cell-free group, the mean total score was 66.3 points before surgery, and 79.2 after surgery, which was significantly improved (P=0.0033). The scores of pain, function and muscle strength were significantly improved (Table II). There was no significant difference in clinical evaluations between the cell transplanted and cell-free control groups before and after surgery.

The improvement of the clinical score was 16.3 in the cell-transplanted group, and 12.9 in the cell-free group, which was not significantly different.

REPAIR TISSUE EVALUATION

The first look operations were performed 6.7 (4–10) weeks after transplantation when the Steinmann's pins were removed.

In three cases of the cell-transplanted with periosteum cover patients the periosteum was partially detached from the original defect and we could observe the repair tissue from there. In the other cases, we could observe the repair tissue when we elevated the periosteums (Fig. 3). The defects were filled with opaque, white to pink tissue which was in the same level as the adjacent normal cartilage and was much softer than the normal cartilage. Histologically, weak and diffuse metachromasia was observed and a small area strong metachromasia existed (Fig. 4). We also observed the periosteum histologically, but no cells that suggested cartilage formation were found.

In four cases of 12 cell-free controls, part of the periosteum was detached and repair tissue was observed from there. In the other cases, we could observe the repair tissue when we elevated the periosteums. The cartilage defect was partially covered by red to yellow tissue with little metachromasie, but the surfaces were irregular.

The second look operations were performed 42 (28–95) weeks after transplantation when the staples were removed. Nine of 12 cell-transplanted, and six of 12 cell-free patients underwent this surgery.

In the cell-transplanted group, cartilage defects were covered with white tissue which was much harder than that observed in the second look operation, but still softer than the normal cartilage (Fig. 5). No periosteum was observed, but in three cases, tissues coming out from the articular surface around the original defect could be observed, which were suggested to be derived from the transplanted periosteum. Histologically, in almost all areas of the sampled tissue metachromasia existed and in some parts hyaline-like cartilage was observed (Fig. 4).

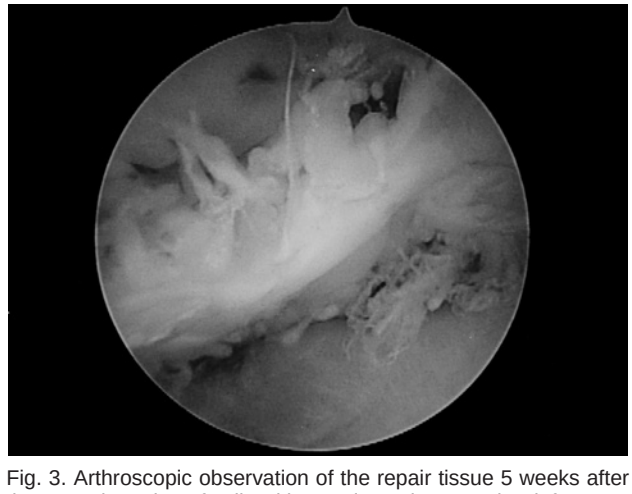

Fig. 3. Arthroscopic observation of the repair tissue 5 weeks after the transplantation of cells with a periosteal cover. The defect was covered with the periosteum, and under the periosteum opaque pink to white repair tissues could be observed.

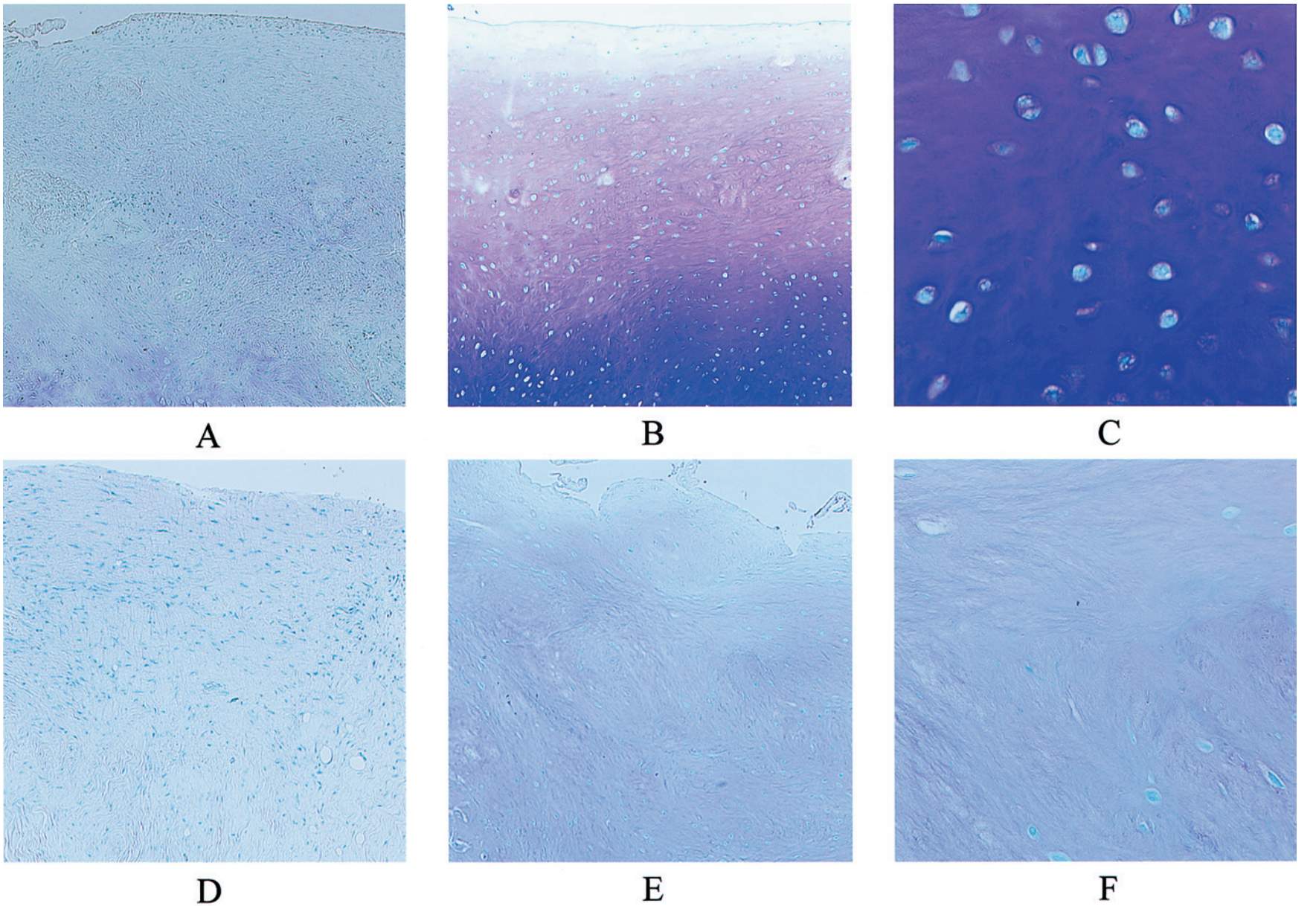

Fig. 4. Histological observation of the repair tissue. (a)–(c) cell-transplanted, (d)–(f) control (abrasion with perforation). (a), (d) 5 weeks, (b), (c) 44 weeks, (e), (f) 43 weeks after the transplantation. (a), (b), (d), (e) ×40, (c) ×200 of (b), (f) ×200 of (e), toluidine blue staining. (a) Diffuse, slight metachromasia was observed. In a small area, round cells with metachromasia were observed, indicating differentiation into chondrocytes. (b) In almost all areas, metachromasia was observed. In the bottom, strong metachromasia was observed. (c) Higher magnification of the bottom area of B. Round cells with strong metachromasia was observed (hyaline cartilage like appearance). (d) Fibrous connective tissue with no metachromasia was observed. (e) Diffuse, slight metachromasia was observed. The surface of the repair tissue was irregular. (f) Higher magnification of E. Low density cells with slight metachromasia were observed (fibrocartilage like appearance).

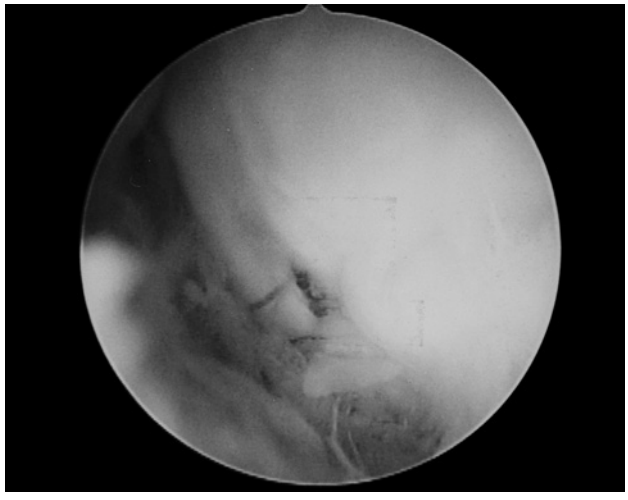

Fig. 5. Arthroscopic observation of the repair tissue 40 weeks after the transplantation of cells with a periosteal cover. The defect was covered with white tissue (right side of the stitches) which was almost the same level as the adjacent normal cartilage.

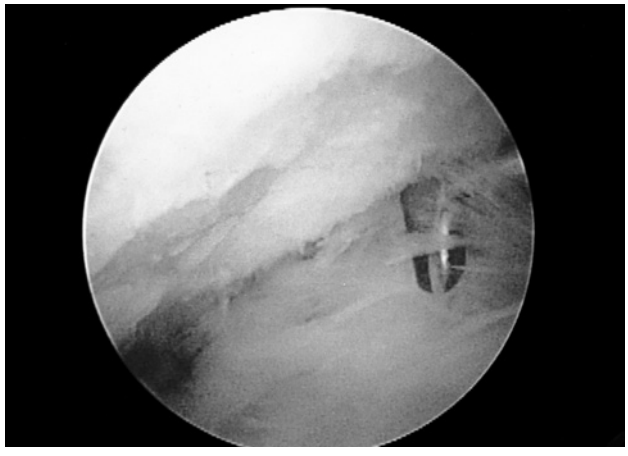

Fig. 6. Arthroscopic observation of the repair tissue 43 weeks after drilling with a periosteal cover. The defect was covered with white material with an irregular surface, and underlying bone was observed in some areas.

In the cell-free group, we observed six cases out of 12 patients in total. We observed three cases of multiple perforation and three cases of abrasion with multiple perforation and there existed no differences between these two on the arthroscopic and histological observations. No periosteum was observed. The defect was covered with white material with an irregular surface and in some areas we could observe underlying bone (Fig. 6). Histologically, metachromasia was weak in the repair tissue (Fig. 4).

The mean arthroscopic grading scores at the first observation were 8.6 points for the cell-transplanted group. In the cell-free group, the scores were 7.2 points for the multiple perforation group and 7.5 points for abrasion with multiple perforation group, which were not significantly different from those of the cell-transplanted group. The mean arthroscopic grading scores at the second observation were 10.4 points for the cell-transplanted group. In the cell-free group, the scores were 7.7 points for the multiple perforation group, which were not significantly different, and 8.3 points for abrasion with multiple perforation group, which were significantly different (P=0.0332) (Table III).

The mean histological grading scores at the first observation were 1.3 points for the cell-transplanted group. In the cell-free group, those were 0.3 points for the multiple perforation group, and 0.5 points for the abrasion with multiple perforation group, which were not significantly different from those of the cell-transplanted group. The mean histological grading scores at the second observation were 5.0 points for the cell-transplanted group. In the cell free groups, those were 2.3 points for the multiple perforation group, which were significantly different (P=0.0449), and 3.0 points for the abrasion with multiple perforation group, which were not significantly different (Table III).

The mean total grading scores at the first observation were 9.8 points for the cell-transplanted group. In the cell-free group, the scores were 7.5 points for the multiple perforation group, and 8.0 points for the abrasion with multiple perforation group, which were significantly different from those of the cell-transplanted group (P=0.0471, 0.0454, respectively). The mean total grading scores at the second observation were 15.4 points for the cell-transplanted group. In the cell free groups, those were 10.0 points for the multiple perforation group, and 11.3 points for the abrasion with multiple perforation group, which were significantly different (P=0.0260, 0.0257, respectively) (Table III).

At the time of high tibial osteotomy, articular cartilage of the poster-medial portion of the medial tibial plateau disappeared, and the subchondral bone was eburnated in all patients. We did nothing for these, and no repair was observed in any cases at any time.

Discussion

REPAIR OF CARTILAGE DEFECTS IN OSTEOARTHRITIC KNEE

Regeneration of articular cartilage defects in osteoarthritic knees was promoted by autologous culture expanded bone marrow mesenchymal cell transplantation. As early as 4–10 weeks after transplantation (first look operation), the defects were covered with white to pink soft tissue, in which metachromasie was partially observed. Twenty-eight to 95 weeks after transplantation (second look operation), the defects were covered with white soft tissue which was much harder than the repair tissue at the first look operation but softer than the surrounding normal cartilage. Histologically, in almost all areas of the sampled tissue metachromasie existed and in some parts hyaline-like cartilage was observed. The arthroscopic and histological grading score of the cell-transplanted group was significantly better than that of the cell-free group at both the first and second look operations.

The mechanism of the promotion of the regeneration of cartilage is unknown. The transplanted chondrocyte progenitor cells may survive and make cartilage. It is also possible that the transplanted cells produce some factors to induce other cells to differentiate into cartilage and that the transplanted cells do not make cartilage. It is impossible to prove whether the transplanted cells survive in the transplanted area in humans.

We have demonstrated that collagen gel provided chondrocytes with a suitable environment in which to synthesize matrix macromolecules and that it fixed cells securely within the defects[16]. Thus, we used collagen gels as delivery vehicles of bone marrow mesenchymal cells.

Table III
Arthroscopic and histological grading score at first and second look operation

	First look			Second look		
		Cell−			Cell−	
	Cell+	per	ab+per	Cell+	per	ab+per
Patient no.	12	6	6	9	3	3
Weeks after surgery	6.3	7.2	6.6	42	46	40
Arthroscopic						
A. Width	2.5±0.7	2.3±0.8	2.3±0.8	2.9±0.3	2.3±1.2	2.3±0.6
B. Thickness	2.8±0.5	2.7±0.5	2.8±0.4	2.8±0.4	2.3±1.2	2.0±0.0
C. Surface regularity	2.2±0.8	1.8±0.8	2.0±0.9	2.8±0.4	1.7±0.6	2.0±0.0
D. Stiffness	0.3±0.5	0.2±0.4	0.2±0.4	1.0±0.5	0.7±0.6	1.0±0.0
E. Color	0.8±0.4	0.2±0.4	0.2±0.4	1.0±0.0	0.7±0.6	1.0±0.0
Subtotal	8.6±1.7	7.2±2.0	7.5±1.5	10.4±1.2	7.7±4.0	8.3±0.6*
Histological						
F. Cell morphology	0.6±0.7	0.2±0.4	0.2±0.4	1.6±0.7	1.0±1.0	1.0±1.0
G. Matrix staining	0.7±0.9	0.2±0.4	0.3±0.5	3.4±0.7	1.3±1.2	2.0±1.7
Subtotal	1.3±1.4	0.3±0.8	0.5±0.8	5.0±1.2	2.3±2.1*	3.0±2.7
Total	9.8±2.0	7.5±2.2*	8.0±0.9*	15.4±1.4	10.0±6.1*	11.3±2.3*

*Significantly different between cell+ and cell− (Mann–Whitney U-test).
Cell+, cell-transplanted; cell−, cell-free.
per, multiple perforation; ab+per, abrasion with multiple perforation.

From the results of this study, we thought that collagen gels worked to some extent. The gels used in this study were from porcine tendon and the sheets were from bovine origin. Although both were mainly type I collagen, we have no idea how the mixing of them influenced the results.

In the knee joint, the alignment of the lower extremity is one of the most important factors for developing OA[17]. For knee OA with various deformity but where the joint is not severely damaged (medial uni-compartmental OA), high tibial osteotomy which corrects the alignment of the lower extremity is frequently performed. The clinical results are reported to be good[17–19]. In this report, the clinical results were as good as those of high tibial osteotomies reported previously; no significant difference in the clinical results of the short follow-up period existed between the cell-transplanted and cell-free groups. The clinical examinations cannot detect difference in the treatment procedure at the short-term follow-up although histological and arthroscopical differences are seen. However, we expect that the regenerated cartilage will make the long-term follow-up results better and reduce the necessity for subsequent total replacement.

The major feature of OA is cartilage erosion, which may lead to eburnation of the underlying subchondral bone. Many attempts have been made to promote cartilage regeneration in the osteoarthritic knee joint. When the mechanical axis of the leg was aligned correctly by high tibial osteotomy, the ulcerate articular cartilage was covered with fibrous and membranous cartilage[20,21]. Pridie reported the effects of joint surface resection by drilling multiple holes into cancellous bone for articular regeneration[22]. The abrasion of eburnated bone (superficial debridement) is reported to be positively effective on the tissue response in humans as compared with the placement of drill holes[23,24]. These methods expose the cancellous bone (spongialization) and promote bleeding from the bone marrow in which progenitor cells and cytokines are contained. These promote tissue regeneration in articular cartilage defects in the osteoarthritic knee joint to some extent, but the repair tissue by exposure of the cancellous bone was reported to degenerate with longer observation[25].

It has been reported that periosteum transplantation was effective in repairing articular cartilage defects[26,27]. It is possible that the periosteum played some role in the repair in our procedure, but we believe that the periosteum did not play an important role because no cartilage formation was observed in the transplanted periosteum as far as we could tell.

BONE MARROW MESENCHYMAL CELL TRANSPLANTATION FOR TISSUE REPAIR

This procedure has many advantages. First, it is easy to obtain autologous cells. We anesthetized partially and aspirated bone marrow blood. There are no side effects of cell collection. The biggest advantage is that we can proliferate cells without losing their capacity to differentiate into cartilage, which is why we can apply this technique in large articular cartilage defects[10]. The average width of the cartilage defect in this study (14 mm×34 mm) represents one of the biggest defects to have been repaired by cell transplantation[3].

In animal experiments, bone marrow mesenchymal cells were reported to differentiate into astrocytes[28] or hepatic oval cells[29], which were not mesenchymal in origin. Thus, bone marrow mesenchymal cells are a potentially useful source of cells for repairing not only mesenchymal tissues but also other dermal tissues.

References

1. Buckwalter JA, Mankin HJ. Articular cartilage repair and transplantation. Arthritis Rheum 1998;41:1331–42.
2. Matsusue Y, Yamamuro T, Hama M. Arthroscopic multiple osteochondral transplantation to the chondral

defect in the knee associated with anterior cruciate ligament disruption. Arthroscopy 1993;9:318–21.
3. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994;331:889–95.
4. Goshima J, Goldberg VM, Caplan AI. The osteogenic potential of culture-expanded rat bone marrow mesenchymal cells assayed in vivo in calcium phosphate ceramic blocks. Clin Orthop 1991;262:298–311.
5. Wakitani S, Saito T, Caplan AI. Myogenic cells derived from rat bone marrow mesenchymal stem cells exposed to 5-azacytidine. Muscle Nerve 1995; 18:1417–26.
6. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, *et al*. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.
7. Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, *et al*. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg 1994;76-A:579–92.
8. Tomita S, Li RK, Weisel RD, Mickle DAG, Kim EJ, Sakai T, *et al*. Autologous transplantation of bone marrow cells improves damaged heart function. Circulation 1999;100(Suppl. II):247–56.
9. Koc ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI, *et al*. Rapid hematopoietic recovery after co-infusion of autologous blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high dose chemotherapy. J Clin Oncol 2000;18:307–16.
10. Caplan AI, Elyaderani M, Mochizuki Y, Wakitani S, Goldberg VM. Principles of cartilage repair and regeneration. Clin Orthop 1997;342:254–69.
11. Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, *et al*. Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. Arthritis Rheum 1986;29:1039–49.
12. Ahlback S. Osteoarthrosis of the knee. A radiographic investigation. Stockholm, Sweden: Karolinska Institute 1968:11–5.
13. Insall JN, Ranawat CS, Aglietti P, Shine J. A comparison of four models of total knee-replacement prostheses. J Bone Joint Surg 1976;58-A:754–65.
14. Outerbridge RE. The etiology of chondromalacia patellae. J Bone Joint Surg 1961;43-B:752–7.
15. Shino K, Nakagawa S, Inoue M, Horibe S, Yoneda M. Deterioration of patellofemoral articular surfaces after anterior cruciate ligament reconstruction. Am J Sports Med 1993;21:206–11.
16. Wakitani S, Kimura T, Hirooka A, Ochi T, Yoneda M, Owaki H, *et al*. Repair of rabbit articular surfaces with allograft chondrocytes embedded in collagen gel. J Bone Joint Surg 1989;71-B:74–80.
17. Insall JN, Joseph DM, Msika C. High tibial osteotomy for varus gonoarthrosis: a long-term follow-up study. J Bone Joint Surg 1984;66-A:1040–8.
18. Matthews LS, Goldstein SA, Malvitz TA, Katz BP, Kaufer H. Proximal tibial osteotomy: factors that influence the duration of satisfactory function. Clin Orthop 1988;229:193–200.
19. Coventry MB, Ilstrup DM, Wallrichs SL. Proximal tibial osteotomy: a critical long-term study of eighty-seven cases. J Bone Joint Surg 1993;75-A:196–201.
20. Odenbring S, Egund N, Lindstrand A, Lohmander LS, Willen H. Cartilage regeneration after proximal tibial osteotomy for medial gonoarthrosis: an arthroscopic, roentgenographic, and histologic study. Clin Orthop 1992;277:210–6.
21. Fujisawa Y, Masuhara K, Shiomi S. The effect of high tibial osteotomy on osteoarthritis of the knee: an arthroscopic study of 54 knee joints. Orthop Clin North Am 1979;10:585–608.
22. Pridie KH. A method of resurfacing osteoarthritic knee joints. J Bone Joint Surg 1959;41-B:618–19.
23. Bert JM, Maschka K. The arthroscopic treatment of unicompartmental gonoarthrosis: a five-year follow-up study of abrasion arthroplasty plus arthroscopic debridement and arthroscopic debridement alone. Arthroscopy 1989;5:25–32.
24. Friedman MJ, Berasi CC, Fox JM, Del Pizzo W, Snyder SJ, Ferkel RD. Preliminary results with abrasion arthroplasty in the osteoarthritic knee. Clin Orthop 1984;182:200–5.
25. Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. J Bone Joint Surg 1976; 58-A:230–3.
26. Hoikka VEJ, Jaroma HJ, Ritsila VA. Reconstruction of the patellar articulation with periosteal grafts: 4-year follow-up of 13 cases. Acta Orthop Scand 1990; 61:36–9.
27. O'Driscoll SW, Keeley FW, Salter RB. Durability of regenerated articular cartilage produced by free autogenous periosteal grafts in major full-thickness defects in joint surfaces under the influence of continuous passive motion: a follow-up report at one year. J Bone Joint Surg 1988;70-A:595–606.
28. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci USA 1999;96:10711–6.
29. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, *et al*. Bone marrow as a potential source of hepatic oval cells. Science 1999;284:1168–70.