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The Effect of the Small and Unstable Autologous Osteochondral Graft on Repairing the Full-Thickness Large Articular Cartilage Defect in a Rabbit Model

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We have investigated the effect of the insufficient autologous osteochondral graft on healing of the large articular cartilage defect using a rabbit model. An osteochondral defect, 7 mm in diameter, was made on the patellar groove of the femoral condyle and repaired with two surgical procedures: Group I, the osteochondral fragment as half as the defect was grafted. The graft was unstable and the size of the graft was smaller than the defect. This is a model of the insufficient autologous osteochondral graft to the large articular cartilage defect; Group II, the defect was left empty. At 2, 4, 12, and 24 weeks after the surgery, the specimens were analyzed macroscopically and histologically. To evaluate the microscopic morphology, a histologic grading scale composed of 5 categories was used. In Group I, although the graft sank a little, a grafted cartilage survived and the reparative fibrous tissue filled the defect covering implanted cartilage. In contrast, in Group II, the defect was only partially covered by fibrocartilaginous tissue with a faintly staining matrix. Throughout the entire observed periods, the scores of the repaired cartilage in Group I are significantly higher than those in Group II. Even the half size of osteochondral graft has an effect to reduce the size of the cartilage defect such as the spacer and leads to better healing compared to the cartilage defect untreated. In case that it is hard to transplant an optimal osteochondral graft because of large cartilage lesion, even the small and unstable osteochondral plug should be transplanted.

Localized articular cartilage lesions associated with traumatic chondral injury, osteochondritis dissecans, and osteonecrosis present a challenging clinical problem. Cartilaginous lesions cause pain and limitation in range of motion of the joint. If left untreated, these lesions can be a precursor to osteoarthritis. In order to prevent development of osteoarthritis, it is important to repair articular cartilage defects by hyaline cartilage with a good congruity of the joint. Many surgical procedures such as, subchondral drilling, abrasion arthroplasty, microfracture, osteochondral graft transplantation, periosteal or perichondrial arthroplasty, and chondrocyte transplantation with collagen gel have been developed (1,2,6,7,10-13). However, cartilage repair is still challenging because the ability of the articular cartilage to repair itself is limited.

Recently, multiple autologous osteochondral transplantation such as mosaicplasty, has been performed and reported with successful clinical results (4,5,8,9). Autologous osteochondral grafts have been reported in treating cartilaginous lesions of the femoral condyle, the tibial plateau, the patella and the talus with less complication at the donor site

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(5,8,9). However, practically, osteochondral graft is sometimes difficult to transplant the osteochondral graft as large as the articular cartilage defect, because harvesting the graft is limited in autologous osteochondral transplantation. Orthopaedic surgeons experience that cartilaginous lesion is too large to repair with several osteochondral grafts in some clinical cases and can not help transplanting the unstable and small osteochondral graft. There has been no basic study examining the effect of the small and unstable autologous osteochondral graft on repairing cartilage defect. Therefore, to understand the effect of the insufficient osteochonral graft on the cartilaginous lesion we made an animal model of osteochondral graft and analyzed the effect of small and unstable graft on the healing status of the large articular cartilage defect.

MATERIALS AND METHODS

The Animal Research Committee of the Kobe University Graduate School of Medicine approved this investigation. Thirty-two skeletally mature female Japanese white rabbits (Kitayama Labes, Nagano, Japan) with a mean weight of 3.2 kg (range, 2.7 to 4.0 kg) were used in this study. General anesthesia was administered using an intravenous pentobarbital sodium solution (30 mg/kg body weight). The rabbits were placed in the supine position and the surgery was performed on the left knee. In each rabbit, the left limb was disinfected and 5 ml of 1 % lidocaine was injected subcutaneously into the medial parapatellar region, where the incision was to be made. A medial parapatellar approach was used to expose the knee joint and the patella was dislocated laterally. The region of the femoral groove, which is in contact with the patella when the knee is flexed at 90 degrees, was selected as the site for the osteochondral defect. A full-thickness cylindrical osteochondral defect (7 mm in diameter and 5 mm in depth) was made through the articular cartilage and into the subchondral bone using the Osteochondral Autograft Transfer System (Arthrex, Naples, Florida, U.S.A.). The rabbits were divided into two groups depending on the mode of the surgical procedure of cartilage repair and sixteen rabbits were used in each group (Fig. 1).

Group I: The removed osteochondral fragment was divided longitudinally into two pieces using a chisel. One half piece of full-thickness osteochondral plug was returned to the site of the original defect without any additional fixation and the other half was discarded. The graft attached the subchondral bone area and partially attached the surrounding normal articular cartilage. This is the model that the defect was repaired partially with the unstable autologous osteochondral graft.

Group Ⅱ: The osteochondral fragment was removed and the full-thickness osteochondral defect was left empty. This is the model in which the defect was left untreated.

In both groups, the cartilage and subchondral debris were removed, and the operative wounds were irrigated with physiological saline before closing the skin. All rabbits were allowed to move freely in their cages after surgery. At 2, 4, 12, and 24 weeks after the surgery, four rabbits at each time point were sacrificed by an intravenous injection of fatal dose of pentobarbital sodium and the femoral condyle was taken and prepared for the macroscopic and histologic evaluations.

For the histological study, the specimens were fixed in 10 % neutral buffered formalin for seven days, decalcified with 0.25 M ethylenediaminetetraacetic acid in phosphate buffered saline at pH 7.5, dehydrated in graded alcohols, and embedded in paraffin wax. Sagittal sections (7μm thick) were then cut and stained with safranin-O fast green, and examined by light microscopy.

Microscopic findings were scored according to the histological grading scale which was

a modification of that described by Wakitani et al (14). The grading system was composed of 5 categories with the highest score of 14 points (TABLE I). The difference in total scores between the two groups at 4, 12, and 24 weeks after the surgery was assessed by the Mann-Whitney U-test for comparison. P-values less than 0.05 were considered to be statistically significant.

TABLE I. Histologic grading scale for cartilage defect.

A. Cell morphology	4	hyaline cartilage
	3	mostly hyaline cartilage
	2	mostly fibrocartilage
	1	mostly noncartilage
	0	noncartilage only
B. Matrix staining with safranin-O	3	normal (compared to host)
	2	slightly reduced
	1	significantly reduced
	0	no staining
C. Surface regularity *	3	smooth (>3/4)
	2	moderate (1/2-3/4)
	1	irregular (1/4-1/2)
	0	severely irregular (<1/4)
D. Thickness of cartilage **	2	>2/3
	1	1/3-2/3
	0	<1/3
E. Integration of donor to host adjacent cartilage	2	both edges integrated
	1	one edge integrated
	0	both edges not integrated
TOTAL MAXIMUM: A-E		14

* Total smooth area of reparative cartilage compared to the whole area of the cartilage defect.

** Average thickness of reparative cartilage compared to that of surrounding cartilage.

RESULTS

In both groups, all the rabbits could move freely in their cages after the second postoperative day. There was no evidence of postoperative wound infection and all the wounds had healed uneventfully.

Macroscopic Findings

Group I: A gap around the implanted plug was detected at 2 and 4 weeks after the surgery (Fig. 2A). The surface of the implanted plug itself appeared intact. At 12 weeks, newly formed reparative tissue was observed around the implanted osteochondral fragment. At 24 weeks, although continuity of articular surface between the implanted osteochondral fragment and the host was observed, the surface of the repaired region appeared to be slightly irregular (Fig. 2B). The color of the surface was not yet uniform.

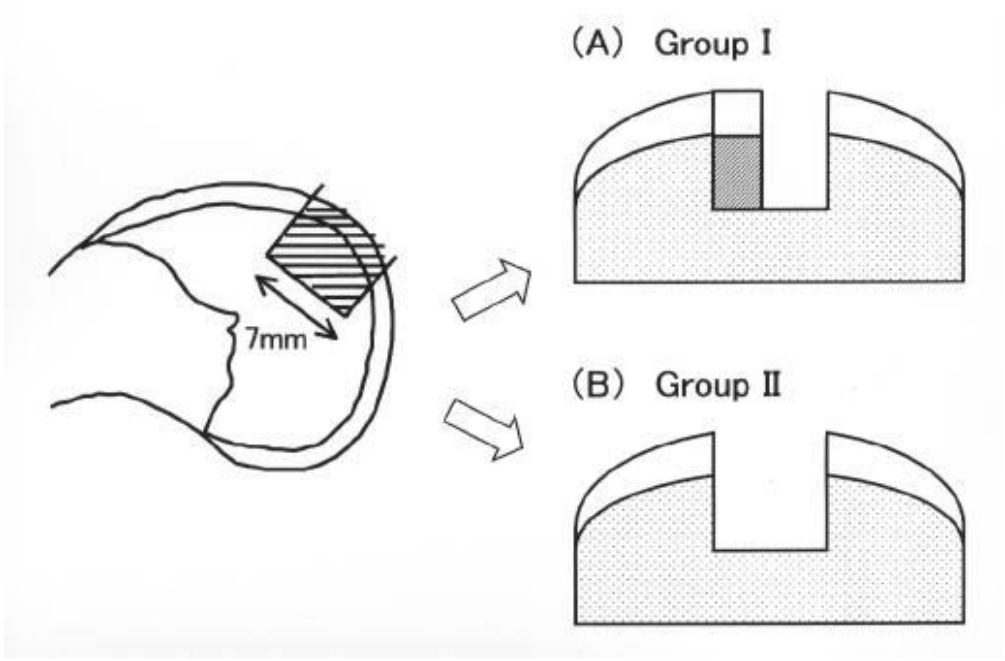

Figure 1. Schematic drawing of surgical procedures. **(A)** Group I: A half of the osteochondral fragment was returned to the defect of the femoral condyle. **(B)** Group Ⅱ: The defect was left empty.

Group Ⅱ: The center of the defect was concave and the margin of the defect was clearly recognized in all of the obtained specimens, from 2 to 12 weeks after the surgery. Although newly formed tissue in the defect was observed at 4 weeks after the surgery, the articular surface of the defect was significantly irregular (Fig. 2C). At 24 weeks, reparative tissue in the defect increased, however, the margin of the defect remained to be recognized (Fig. 2D). The surface of the operated region was still irregular compared with the articular surface of the intact femoral condyle and its appearance was not glossy.

Histological Findings

Group I: At 2 and 4 weeks after the surgery, the implanted osteochondral plug had united in the subchondral bone area. The implanted osteochondral plug subsided a little and the fibrous tissue, which was stained with safranin-O fast green, filled the gap surrounding the implanted osteochondral fragment (Fig. 3A). At 12 and 24 weeks after the surgery, the implanted fragment had subsided. Newly formed fibrous tissue, which was stained with safranin-O fast green moderately, covered the surface of the defect and the implanted cartilage. Continuity of the articular surface was observed between the newly formed fibrous tissue and the host (Fig. 3B). The reparative tissue, which filled the defect and covered the implanted cartilage, was stained with safranin-O fast green faintly compared with the surrounding host cartilage.

Group Ⅱ: At 4 weeks after the surgery, the subchondral bone at the bottom of the defect had been regenerated, however, no cartilaginous tissue was observed (Fig. 4A). At 24 weeks after the surgery, the subchondral bone had been almost remodeled, however, the defect was partially covered by thin fibrous tissue by faintly stained with safranin-O fast green. The reparative tissue had extended to almost the same level of the adjacent normal articular cartilage (Fig. 4B). However, no reparative hyaline cartilaginous tissue was observed.

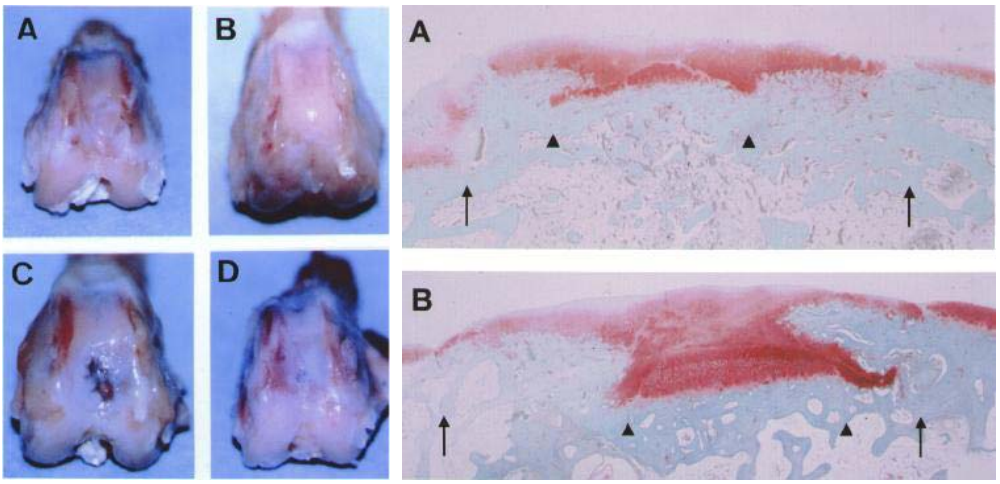

Figure 2. (left) Macroscopic appearances of the femoral condyle at 4 and 24 weeks after the surgery. **(A)** 4 weeks in Group I. The defect was filled with a half of the osteochondral fragment and newly formed tissue. **(B)** 24 weeks in Group I. Continuity of articular surface between the implanted osteochondral fragment and the host was observed. **(C)** 4 weeks in Group Ⅱ. Although newly formed tissue was observed in the defect, the articular surface of the defect was significantly irregular. **(D)** 24 weeks in Group Ⅱ. Reparative tissue in the defect increased, however, the center of the defect was still concave.

Figure 3. (right) Histological findings of the sagittal section of the femoral condyle in Group I. A section was stained with safranin-O fast green (original magnification X10) (the arrow heads indicate the implanted osteochondral graft and the arrows indicate the osteochondral defect). **(A)** Postoperative 4 weeks: The implanted osteochondral fragment subsided a little and the fibrous tissue, stained with safranin-O fast green, was observed around the implanted osteochondral. **(B)** Postoperative 24 weeks: The implanted graft subsided deeper, however, the structural property of cartilage remained normal.

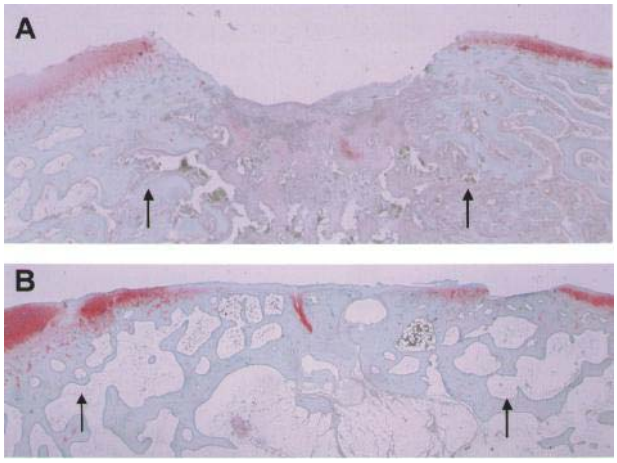

Figure 4. Histological findings of the sagittal section of the femoral condyle in Group Ⅱ. A section was stained with safranin-O fast green (original magnification X10) (the arrows indicate the osteochondral defect). **(A)** Postoperative 4 weeks: No cartilagenous tissue was observed in the defect. **(B)** Postoperative 24 weeks: The subchondral bone had been almost remodeled and the defect was partially covered by thin metaplastic cartilagenous tissue.

Histologic Scoring of the Reparative Tissue

In Group I, the total score was 5, 5, and 6 points at 4, 12, and 24 weeks after the surgery (TABLE Ⅱ). In Group Ⅱ, the score remained less than 3 points and remarkably low compared with Group I throughout the entire observation periods. The total score of Group I was significantly higher than that of Group Ⅱ at each of the time-point after the surgery, using the Mann-Whitney U-test (Fig. 5).

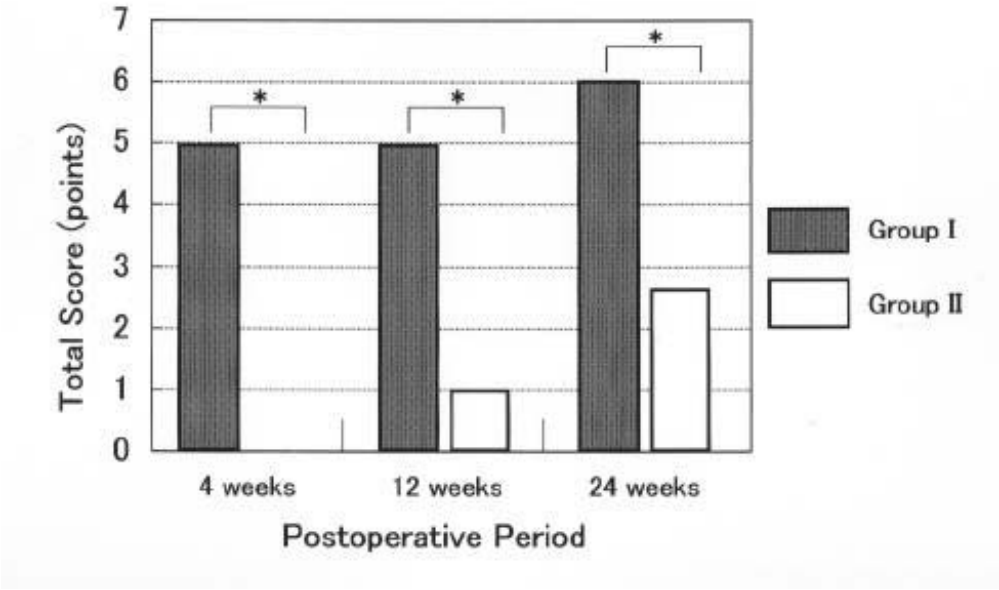

Figure 5. Total score of histological evaluation. The total score of Group I was significantly higher than that of Group Ⅱ through the entire postoperative period (* $p<0.05$).

TABLE Ⅱ. Results of histologic grading scale.

Time after surgery	Histologic grading scale (Median value) A	B	C	D	E	Total score
Group I						
4W	2	1	1	1	0	5
12W	2	1	1	1	0	5
24W	2	1	1	1	1	6
Group II						
4W	0	0	0	0	0	0
12W	0	1	0	0	0	1
24W	1	1	0	0	0	2

DISCUSSION

In the present study, Group I is the surgical model that the cartilage defect was repaired with the insufficient unstable graft. Because the osteochondral graft was not transplanted precisely, the graft subsided and newly formed fibrous tissue occupied the space around and over the graft. Extracellular matrix of these newly formed tissue was stained with safranin-O. In Group Ⅱ in which the defect was left untreated, the surface of the reparative tissue had extended to the level corresponding to that of the adjacent host articular cartilage at 24 weeks after the surgery. However, no regeneration of hyaline-like cartilaginous tissue was observed as has been reported previously (3).

Autologous osteochondral transplantation has several advantages, such as, reliability of the bone union, a high survival rate of the grafted articular cartilage, and no threat of disease transmission (9). Osteochondral grafts with a bone plug can be stabilized at the site of transplantation more quickly than grafts without a bone plug or cultured chondrocyte transplantation, because early bone union can be obtained in the subchondral area (4). The cartilage grafted with a bone plug survived for a long term compared with the cartilage without a bone plug because of the increased stability and better supply of nutrition. When autologous osteochondral transplantation is performed in the weight bearing area, graft subsidence can be one of the possible complications (4). Therefore, it is considered that the size of osteochondral plug should be match to the cartilaginous lesion, to obtain the excellent result of osteochondral transplantation. However, in clinical practice of the surgery, many orthopaedic surgeons experience that cartilaginous lesion is too large to transplant optimal autologous osteochondral plug.

In the macroscopic findings at postoperative 24 weeks in Group I, newly formed tissue filled the defect and smooth continuity of cartilage surface was observed. In the microscopic findings, histological score of Group I was better than that of Group Ⅱ throughout the entire postoperative periods. In case of osteochondral transplantation of extremely small osteochondral graft, the graft might progress to necrosis and the histologic scoring might approximate to that Group Ⅱ. In this study, we could not mention that the smallest size of the graft to obtain a satisfactory clinical result. However, it is suggested that the autologous osteochondral grafts with small unstable graft has the effect of reducing the size of the large cartilage defect as a spacer resulting in a better score.

Needless to say, for obtaining excellent clinical results, it is important to implant an osteochondral graft as large as the defect. However, if the surgeons recognize that it is hard to transplant the graft as large as the cartilage lesion in the operation room, even the small and unstable osteochondral plug should be transplanted. We conclude that the graft still has a beneficial effect on repair of the cartilaginous lesion by reducing the size of the defect as a spacer even if the graft is not large enough to fill the defect. Further investigations of histological, mechanical, and biological properties of the cartilage grafted insufficiently are necessary to verify the long-term effects of osteochondral transplantation.

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