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THE EFFECT OF GRAFT SIZING ON OSTEOCHONDRAL  
TRANSPLANTATION

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1 ABSTRACT

2 **Purpose:** The goal of this study is to analyze the effect of the graft sizing on  
3 the histological property of articular cartilage in autologous osteochondral  
4 transplantation.

5 **Type of study:** Histological analysis using an animal model.

6 **Materials and Methods:** Eighteen skeletally mature female Japanese white  
7 rabbits were used in this study and divided into two groups based on the  
8 surgical procedure. **Group I:** A cylindrical fragment (7 mm in diameter and  
9 5 mm in depth) was harvested at the femoral condyle using the  
10 Osteochondral Autograft Transfer System (Arthrex, Florida, U.S.A.), then  
11 precisely returned to the defect from which the fragment was originally  
12 harvested. This surgical model supposes that the articular surface  
13 geometry of the osteochondral graft exactly match that of the cartilage  
14 lesion; however, the osteochondral graft is not the same size as the defect  
15 in this model because of the surrounding gap which corresponds to the  
16 blade thickness of the chisel used to take the graft. **Group II:** A 1 mm  
17 larger osteochondral fragment (8 mm in diameter and 5 mm in depth)

1 taken from the contralateral femoral condyle was transplanted to the  
2 osteochondral defect made as in Group I achieving a tight fit. Specimens  
3 were stained with Safranin-O fast green and analyzed microscopically at 4,  
4 12 and 24 weeks after surgery.

5 **Results:** In Group I, histological examination revealed an increase in  
6 cartilage thickness and cell density during the implantation period. Round  
7 and polygonal hypertrophic clusters of chondrocytes with cytoplasmic  
8 vacuoles were observed. By contrast, in Group II, thickness of the articular  
9 cartilage was almost the same as that of the normal adjacent cartilage  
10 with no significant change being observed.

11 **Conclusions:** It is suggested that sizing of the implanted osteochondral  
12 fragment play an important role in preserving the histological properties  
13 of cartilage.

14 **Key words:** Cartilage repair, Autologous osteochondral transplantaion, Graft  
15 sizing, Histology

# 1 INTRODUCTION

2 Articular cartilage defects associated with traumatic chondral injury,  
3 osteochondritis dissecans, and osteonecrosis may be repaired by a number  
4 of surgical procedures such as subchondral drilling, abrasion arthroplasty,  
5 osteochondral grafts, periosteal or perichondrial grafts, chondrocyte  
6 transplantation with collagen gel, and autologous osteochondral  
7 transplantation.<sup>1-9</sup>

8 Autologous osteochondral transplantation system is commercially  
9 available to clinics, however, histological findings regarding the implanted  
10 cartilage remain controversial.<sup>9-15</sup> Previous reports of both  
11 post-transplantation biopsies and animal experiments reveal  
12 maintainance of cell viability without significant remodeling on gross and  
13 light microscopic examination of the transplanted cartilage.<sup>9-12</sup> It is  
14 understood that chondrocytes survive after osteochondral transplantation  
15 via diffusion of joint fluid without requiring a blood supply for nutrition.

16 In contrast, hypercellularity or partial degenerative changes of the  
17 transplanted cartilage have also been reported after autologous

1 osteochondral transplantation.<sup>13,14</sup> In a previous report, we have described  
2 histological changes occurring in the implanted cartilage, in the case of an  
3 osteochondral graft taken from the femoral condyle and returned to its  
4 original site.<sup>15</sup> The surgical model in that study was selected so that the  
5 surface geometry of the graft exactly matched the surface of the lesion.  
6 However, the osteochondral graft was not the same size as the defect in  
7 that model because of the blade thickness of the chisel used to take the  
8 graft, resulting in an undersized graft compared to the defect.  
9 We thus hypothesized that graft sizing may effect histological changes in  
10 articular cartilage in osteochondral transplantation. The purpose of this  
11 study is to analyze the effect of graft sizing on the histological changes of  
12 the implanted cartilage.

13

## 14 MATERIALS AND METHODS

15 The Animal Research Committee of the Kobe University Graduate School  
16 of Medicine reviewed the study protocol and approved this investigation.  
17 Eighteen skeletally mature female Japanese white rabbits (Kitayama

1 Labes, Nagano, Japan) with a mean weight of 3.2 kg (range, 2.7 to 4.0 kg)  
2 were used in this study. At surgery, general anesthesia was administered  
3 using an intravenous pentobarbital sodium solution (30 mg/kg body  
4 weight). The rabbits were placed in the supine position and the surgery  
5 was performed on the left knee. In each rabbit, the left limb was  
6 disinfected, and 5 ml of 1 % lidocaine was injected subcutaneously into the  
7 medial parapatellar region, where the incision was to be made. A medial  
8 parapatellar approach was used to expose the knee joint. The region of the  
9 trochlear of the femoral condyle, which is in contact with the patella when  
10 the knee is flexed at 90 degrees, was selected as the site for the  
11 osteochondral defect. The rabbits were divided into two groups depending  
12 on the mode of surgical procedure for cartilage repair, with each group  
13 composing 9 rabbits (Figure 1).

14 **Group I:** A full-thickness cylindrical defect (7 mm in diameter and 5 mm in  
15 depth) was created using the Osteochondral Autograft Transfer System  
16 (Arthrex, Inc., Naples, Florida, U.S.A.). The osteochondral fragment was  
17 removed and precisely returned to its original site. This surgical model is

1 that the surface geometry of the graft exactly matches the surface of the  
2 cartilage lesion, however the transplanted graft is undersized compared  
3 with the lesion because of the surrounding gap which corresponds to the  
4 blade thickness of the chisel used to take the graft.

5 **Group II:** A full-thickness cylindrical defect (7 mm in diameter and 5 mm  
6 in depth) was grafted with a 1 mm oversized osteochondral donor graft (8  
7 mm in diameter and 5 mm in depth) harvested from the contralateral  
8 femoral condyle. In this model the oversized graft is transplanted to the  
9 cartilage lesion.

10 At 4, 12, and 24 weeks postoperatively, three rabbits from each group were  
11 euthanized with an intravenous injection of a fatal dose of pentobarbital  
12 sodium and the femoral condyle was taken from the knee, then prepared  
13 for macroscopic and histologic evaluation.

14 For the histological study, the specimens were fixed in 10 % neutral  
15 buffered formalin for seven days, decalcified with 0.25 M  
16 ethylenediaminetetraacetic acid in phosphate buffered saline at pH 7.5,  
17 dehydrated in 70, 80, 90 and 100 % alcohol, and embedded in paraffin wax.



1 Sagittal sections ( $7\text{ }\mu\text{ m}$  thick) were stained with safranin-O fast green, and  
2 examined by light microscopy.

3

#### 4 RESULTS

5 All the rabbits moved freely in their cages by the second postoperative day.

6 No evidence of postoperative infection at the wound site was observed, and  
7 all the wounds healed uneventfully.

8 On macroscopic findings, four weeks after surgery, a gap around the  
9 implanted osteochondral fragment was clearly identified in both groups. At  
10 12 and 24 weeks after surgery, the gap around the graft had filled, the  
11 boundary line of the graft had become unclear, and the articular surface  
12 between the implanted osteochondral fragment and the host cartilage was  
13 smooth and continuous in both groups.

14 In Group I, histological examination at 4 weeks revealed that the  
15 transplanted graft had united at the subchondral bone area, however, the  
16 osteochondral fragment had sunk and fibrous tissue covered the fragment  
17 at the proximal and articular site of the transplanted fragment. The layer

1 of the grafted cartilage was thicker than that of the normal host cartilage.  
2 At high magnification, cell density was higher and chondrocytes with  
3 intracellular cytoplasmic vacuoles were observed. These histological  
4 changes of increase in cartilage thickness and cell density as well as  
5 chondrocytes with intracellular cytoplasmic vacuoles continued to be  
6 observed at 8 and 24 weeks after surgery (Figure 2A and 2B).

7 In Group II, the transplanted graft had united at the subchondral bone  
8 area and no sinking of the osteochondral fragment was observed when  
9 examined at 4 weeks. Although a cleft around the osteochondral fragment  
10 was detected, histological changes in the grafted articular cartilage were  
11 not observed throughout the entire postoperative period when compared  
12 with the host cartilage. Thickness, cell density, and morphology of the  
13 transplanted cartilage appeared to be normal (Figure 2C and 2D).

14

## 15 DISCUSSION

16 In Group I, cartilage thickness increase and fibrous tissue covered the  
17 graft at the proximal site. By contrast, in Group II, no significant change

1 was observed in the transplanted graft. It is suggested that fibrous tissue  
2 have covered the transplanted graft because the transplanted  
3 osteochondral fragment sunk at the cartilage defect in Group I.

4 The remodeling process of the osteochondral graft in autologous  
5 osteochondral transplantation has not been well clarified. Since healing  
6 and incorporation of the graft are influenced by various factors, results  
7 reported have varied depending on the respective biological and  
8 biomechanical environments.<sup>9-15</sup>

9 The repair and regeneration process of nonvascularized autologous  
10 cancellous bone graft is similar to the repair and regeneration process in  
11 osteonecrosis of cancellous bone.<sup>16</sup> In this process vascular ingrowth and  
12 progenitor mesenchymal cell invades after cell death, an osteoblastic  
13 appositional new bone forms onto the dead trabeculae, and remodeling of  
14 the trabeculae to mature pattern occurs.<sup>16</sup>

15 Although long bone fracture typically exhibits endochondral bone  
16 formation and membranous bone formation during healing, fractures with  
17 rigid compression plating and external fixation heal without a visible

1 callus by direct haversian remodeling, called primary bone healing.<sup>16</sup>  
2 Because the oversized osteochondral graft was transplanted in Group II,  
3 increased graft stability may be obtained in Group II compared with  
4 Group I. Therefore, the repair process of the transplanted osteochondral  
5 fragment in Group II is considered to be different to that of Group I.  
6 Autologous osteochondral transplantation is clinically a useful surgical  
7 treatment for articular cartilage lesions of the femoral condyle.<sup>5,8,9</sup> Several  
8 autologous osteochondral transplantation systems are commercially  
9 available. Most of these systems recommend that a slightly oversized  
10 osteochondral fragment should be transplanted to achieve the tight fit of  
11 the fragment. The present study suggests that sizing of the implanted  
12 osteochondral fragment influences its healing process and plays an  
13 important role in preserving the histological properties of cartilage.

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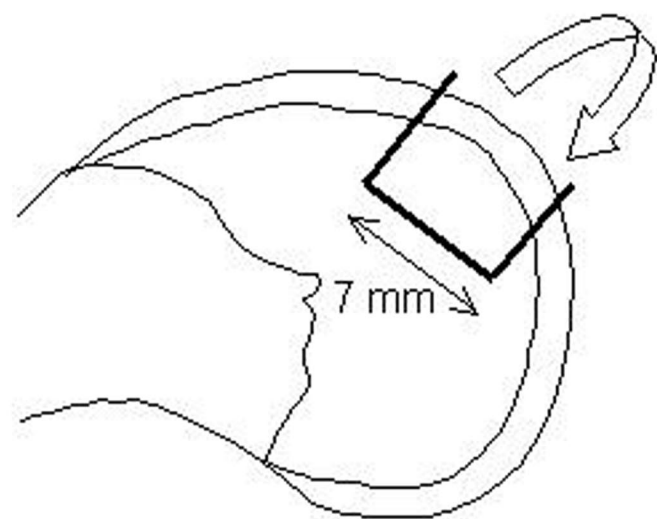
## LEGENDS

**Figure 1.** Surgical procedures. **(A)** Group I: Full-thickness cylindrical defect (7 mm in diameter and 5 mm in depth) was made and the osteochondral fragment was removed and precisely returned to its original site. **(B)** Group II: Osteochondral graft (8 mm in diameter and 5 mm in depth) was taken from the contralateral femoral condyle and transplanted to the defect (7 mm in diameter and 5 mm in depth).

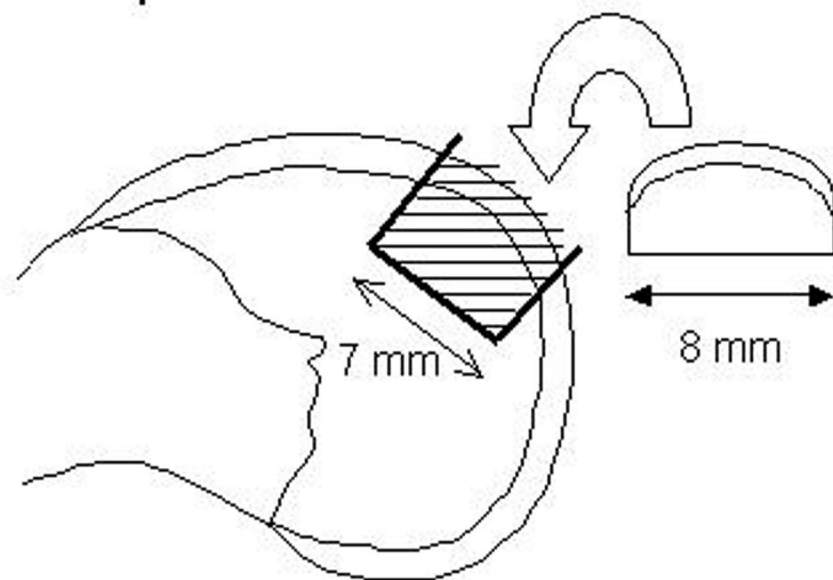
**Figure 2.** Histological findings of saggital sesction of the femoral condyle at postoperative 24 weeks stained with Safranin-O fast green. **(A)** Group I: The layer of the grafted cartilage was thicker compared with the layer of the normal host cartilage. Arrows indicate the margins of the graft. (original magnification  $\times 30$ ). **(B)** Group I: An increase in the numbers of chondrocytes and intracytoplasmic vacuoles was observed in chondrocytes. (original magnification  $\times 300$ ). **(C)** Group II: Thickness of cartilage, cell density, and morphology of the transplanted cartilage appear similar to the normal host cartilage. Arrows indicate the margins of the graft.

(original magnification  $\times 30$ ). **(D)** Group II: Thickness of cartilage, cell density, and morphology of the transplanted cartilage appear well preserved. (original magnification  $\times 300$ ).

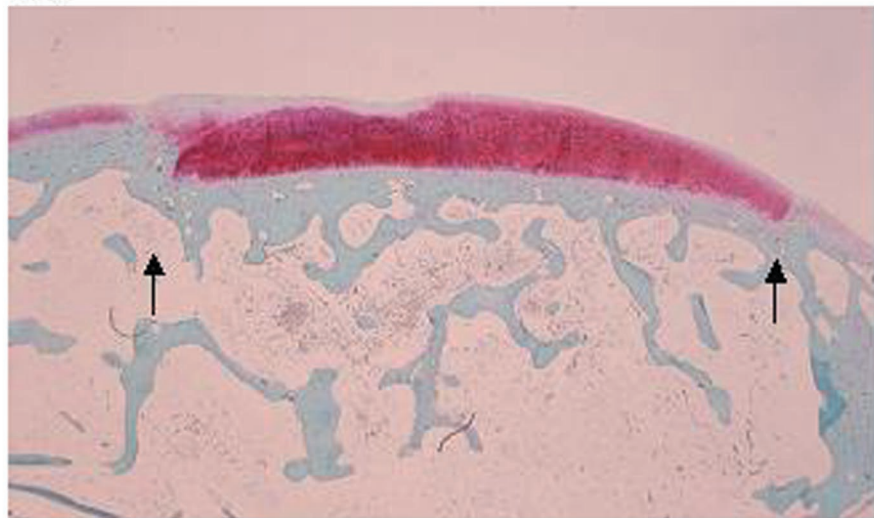
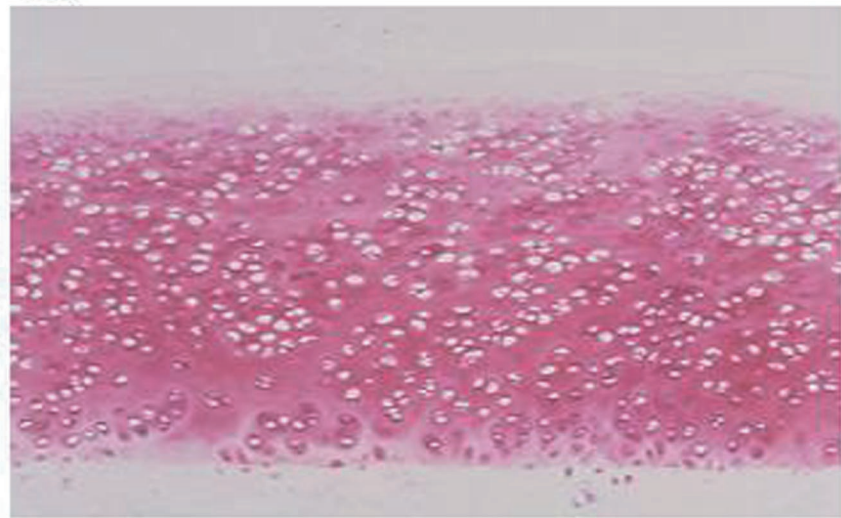
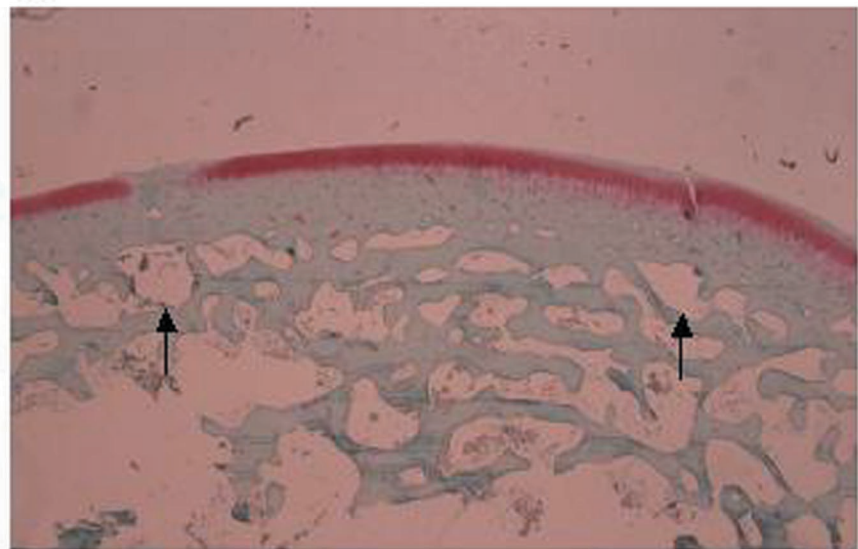
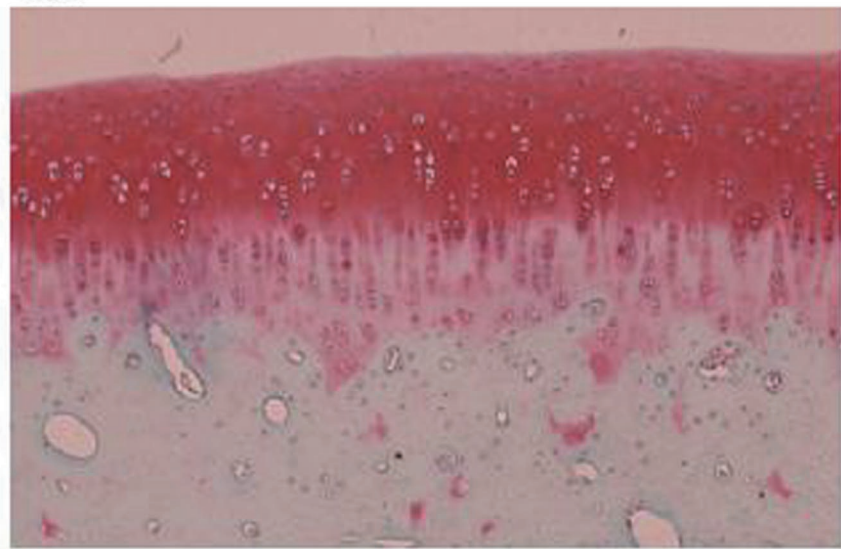
Group I



Group II



**Figure 1**

**A****B****C****D**

**Figure 2**