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The Structural Properties of an Osteochondral Cylinder Graft-Recipient Construct on Autologous Osteochondral Transplantation

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ABSTRACT

Purpose: The purpose of this study was to investigate the changes of structural properties of an osteochondral cylinder graft-recipient construct after autologous osteochondral transplantation.

Type of study: Mechanical analysis using an animal model.

Materials and Methods: A full-thickness cylindrical osteochondral defect (5 mm in diameter and 3 mm in depth) was made on the femoral condyle of a mature female Japanese white rabbit using the Osteochondral Autograft Transfer System (OATS: Arthrex, Naples, FL). The defect was repaired with an osteochondral plug (6 mm in diameter and 3 mm in depth) taken from the contralateral femoral condyle using the OATS. The implanted osteochondral grafts were evaluated immediately after surgery and at postoperative weeks 1, 3, 8, and 12. The stiffness of articular cartilage was analyzed using a tactile sensor system (AXIOM Co., Ltd., Fukushima, Japan), which measures stiffness based on changes in resonance frequency when a vibrating tactile sensor touches articular cartilage. The specimens were stained with hematoxylin and eosin and serial sections were examined microscopically.

Results: The cartilage stiffness of the graft immediately after surgery was

107695.1 N/m^2, which was not statistically different from the normal cartilage stiffness (100027.5 N/m^2). The stiffness at postoperative weeks 1, 3, 8, and 12 was 95386.8, 92899.3, 95969.8, and 104683.7 N/m^2, respectively. The stiffness at postoperative weeks 1, 3, and 8 was significantly lower than the normal cartilage stiffness and the stiffness at postoperative week 12 was the same as normal cartilage. A new bone formation with an increase of bone trabeculae between the osteochondral cylinder graft and the recipient was observed at postoperative week 1, 3, and 8. Thereafter, at postoperative week 12, bone trabeculae decreased to the same level as observed in a normal model due to the progress of bone remodeling.

Conclusions: The stiffness of articular cartilage of the osteochondral graft was normal at the time the graft was initially placed and at postoperative week 12. However, the stiffness at postoperative weeks 1, 3, and 8 was lower than the normal cartilage stiffness.

Clinical Relevance: Care should be taken when making the rehabilitation program at an early phase after osteochondral transplantation.

Key words: Cartilage Repair, Osteochondral Graft, Mechanical Property

INTRODUCTION

Many surgical procedures have been developed for the repair of injured articular cartilage, such as subchondral drilling, abrasion arthroplasty, osteochondral grafts, periosteal or perichondrial grafts, and chondrocyte transplantation with collagen gel.[1-8] Autologous osteochondral transplantation has been gaining acceptance as it has several advantages, including reliable bony union, a high survival rate of the grafted articular cartilage, and no threat of disease transmission.[3,4,7]

Both histologic and mechanical analyses are important for evaluating the effects of autologous osteochondral transplantation. Histologic analysis of the transplanted osteochondral graft indicates that in an optimal autologous osteochondral transplantation, osteochondral grafts are incorporated into defects to retain the integrity of hyaline cartilage and cancellous bone, and to maintain the congruity of the articular surface in weight-bearing areas.[3,4,9] In contrast, changes in the mechanical properties of the transplanted osteochondral graft throughout the postoperative period are not well understood and the optimum time to start weight-bearing exercise is controversial. Hangody et al. suggested that a 2 to 3-week non-weight-bearing period was sufficient.[4] Wang recommended that patients remain in a non-weight-bearing condition for 12 weeks.[10] Therefore, we examined the sequential changes of the structural and histological properties of an osteochondral cylinder graft-recipient construct from immediately after surgery until the graft was incorporated into the defect, using a rabbit osteochondral graft model, in an attempt to determine the optimum time to start weight-bearing and the rehabilitation program after an osteochondral graft.

MATERIALS AND METHODS

Surgical Procedures

This investigation was approved by the Animal Research Committee of Kobe University Graduate School of Medicine. Skeletally-mature female Japanese white rabbits (n = 25; Kitayama LABES, Nagano, Japan) were used in the study. Mean weight was approximately 3.2 kg (range, 2.7 - 3.5 kg). General anesthesia was administered using an intravenous pentobarbital sodium solution (30 mg/kg body weight). The rabbits were placed in a supine position, and the surgery was performed on the right knee. In each rabbit, the right limb was disinfected, and 3 ml of 1% lidocaine was injected subcutaneously into the medial parapatellar region.

A medial parapatellar approach was used to expose the knee joint, and the patella was laterally dislocated. The region of the femoral groove, which contacted with the patella when the knee was flexed at 90°, was selected as the site for the osteochondral defect. To make the cartilage defect, a full-thickness cylindrical osteochondral fragment (5 mm in diameter and 3 mm in depth), which included the articular cartilage and the subchondral bone, was taken using the Osteochondral Autograft Transfer System (OATS: Arthrex, Naples, FL). To repair this defect, an osteochondral plug (6 mm in diameter and 3 mm in depth) taken from the left femoral condyle using the OATS was transplanted. The implanted osteochondral grafts were then evaluated mechanically immediately after surgery and at postoperative weeks 1, 3, 8, and 12. At each time point after mechanical analysis, the specimen was examined histologically. Five rabbits were used at each time point.

Mechanical Analysis

To analyse the structural properties of an osteochondral cylinder graft-recipient construct, the stiffness of the articular cartilage was measured with a tactile sensor system (AXIOM Co., Ltd., Fukushima, Japan)[11-13] developed to detect the stiffness of a material. Each material has its own resonance frequency and when a tactile sensor vibrating at its own frequency touches an object, the resonance frequency of the object shifts. The difference between the frequencies under non-touching and touching conditions depends on the stiffness of the objects, and the stiffness of the material can be estimated by monitoring the change in frequency.[11] The tactile sensor system consists of a sensor probe, an amplifier, and a filter.

In this study, the tactile sensor probe had a hemispheric tip, 2 mm in diameter. The displacement rate of the probe and the maximum displacement were set at 1 mm/s and 0.1 mm, respectively. The change in frequency (Δf) of normal articular cartilage was measured before surgery as a control and the Δf of the articular cartilage of the transplanted graft was measured at each time point after surgery (Fig 1). The stiffness of articular cartilage was calculated from Δf using the following calibration formula: stiffness (N/m^2) = 233.2 x Δf (Hz) +104948.0 (where $R^2=0.91$).[12] The stiffness of articular cartilage at each time point after surgery was compared to the stiffness of normal articular cartilage. Statistical significance was calculated using unpaired t-tests. A p value of less than

0.05 was considered statistically significant.

Histologic Analysis

The specimens were fixed in 4% paraformaldehyde phosphate buffer solution, decalcified with 0.25 mol/L ethylenediaminetetraacetic acid in phosphate buffered saline at pH 7.5, dehydrated in graded alcohol solutions, and embedded in paraffin wax. Sagittal sections (7 μm thick) were cut and stained with hematoxylin and eosin. Serial sections were taken from each specimen and 50 sections per specimen were examined microscopically. To understand the histological changes after surgery, the normal femoral condyle was examined and compared as a control.

RESULTS

Mechanical Analysis

Normal articular cartilage of the femoral condyle showed changes in frequency (Δf) of -21.1 ± 1.7 Hz. Immediately after surgery, the average Δf was 11.78 ± 49.8 Hz. At postoperative weeks 1, 3, 8, and 12, the average Δf was -41.0 ± 11.5, -51.7 ± 16.1, -38.5 ± 9.3, and -1.1 ± 14.2 Hz, respectively. The data was calibrated with the calibration formula and a stiffness value was obtained (Fig 2). The average stiffness of normal articular cartilage was 100027.5 ± 396.4 N/m^2. Immediately after surgery, the average stiffness of articular cartilage of the transplanted graft was 107695.1 ± 11610.1 N/m^2, thus not significantly different from the normal cartilage stiffness. At postoperative weeks 1, 3, and 8, the average stiffness of articular cartilage of the transplanted graft was 95386.8 ± 2689.4, 92899.3 ± 3748.2, and 95969.8 ± 2157.1 N/m^2, respectively. These data were significantly lower than the normal cartilage stiffness; the stiffness at postoperative week 3 was the lowest. The average stiffness at 12 weeks after surgery, however, was 104683.7 ± 3311.5 N/m^2, which was not statistically different from the normal cartilage stiffness.

Histologic Analysis

The representative histological findings after surgery and the normal histological findings of the femoral condyle are presented in Figure 3.

The gap in the articular cartilage surface between the graft and the recipient was clearly detected in all rabbits, immediately after surgery (Fig 3 A) and 1 week after surgery (Fig 3 B). At postoperative week 3 (Fig 3 C), the gap between the graft and the recipient became unclear. At postoperative week 8 (Fig 3 D), continuity of the articular cartilage surface were observed in all specimens. At postoperative week 12 (Fig 3 E), continuity of the articular cartilage surface was better than that observed at postoperative week 8. The discontinuity of the articular surface gradually improved from 3 weeks to 12 weeks after surgery. There was no remarkable change in the cells of articular cartilage throughout the entire postoperative period.

At the subchondral bone area, discontinuity of bone trabeculae was observed at the border between the osteochondral graft and the recipient immediately after surgery (Fig 3 A). An increase in bone trabeculae at the border between the osteochondral cylinder graft and the recipient was observed at postoperative week 1, 3, and 8 (Fig 3 B, C, D). However, bone trabeculae at postoperative week 12 (Fig 3 E), which was almost the same as normal (Fig 3 F), showed a decrease in bone trabeculae compared with those at postoperative week 1, 3, and 8.

DISCUSSION

We present sequential changes in the structural properties of an osteochondral cylinder graft-recipient construct, measuring the stiffness of articular cartilage with a tactile sensor from immediately after surgery to postoperative week 12. Immediately after surgery, the stiffness of articular cartilage of the transplanted graft was not significantly different from normal cartilage. However, the stiffness at postoperative weeks 1, 3, and 8 was significantly lower than that of normal cartilage and the stiffness at postoperative weeks 3 was the lowest. At postoperative week 12, the stiffness of the graft was greater than that at postoperative weeks 1, 3, and 8, and was at the same level as normal articular cartilage.

The mechanical properties of articular cartilage have been analyzed in various autologous osteochondral transplantation models and evaluated at various periods after surgery. Kuroki et al. examined the mechanical effects of autologous osteochondral grafting on articular cartilage in a porcine model using an ultrasonic measurement system immediately after surgery and suggested that osteochondral graft surgery did not affect stiffness, surface irregularity, or thickness of the plug.[14] Whiteside et al. reported that average push-in and pull-out fixation strength at postoperative day 7 decreased 44% compared with the strength immediately after surgery.[15] Nam et al. reported that the stiffness at 6 weeks after osteochondral transplantation was lower than that of normal cartilage in a biomechanical evaluation of articular cartilage of the transplanted graft using a rabbit model.[16] Lane et al. assessed biochemical and biomechanical changes in a goat osteochondral autograft model.[17] They described the stiffness of articular cartilage at 12 weeks after surgery as greater than in normal cartilage with maintenance of chondrocyte cellular viability, and expressed concern regarding the increased trabecular mass in the healing subchondral area. We have demonstrated that the structural properties of an osteochondral cylinder graft-recipient construct immediately after surgery were not significantly different from normal cartilage and that the structural properties of an osteochondral cylinder graft-recipient construct at postoperative weeks 1, 3, and 8 were significantly lower than normal cartilage. Our data are consistent with previous reports. These statements might have important implications for considering the best time to start weight-bearing or range of motion exercise after autologous osteochondral transplantation surgery, of which clinical decisions remain controversial.[4,10]

The structural properties of an osteochondral cylinder graft-recipient at the time the graft was initially placed in the defect were the same as normal cartilage although the gap between the graft and the recipient was detected. It is speculated that the initial stability of the graft was obtained because the size of the graft (6 mm in diameter) was larger than of the defect (5 mm in diameter).

By contrast, the structural properties of an osteochondral cylinder graft-recipient at postoperative weeks 1, 3, and 8 were significantly lower than normal cartilage. At postoperative week 1, the gap at the articular surface between the transplanted graft and the recipient was observed and bone trabeculae at the subchondral area increased. It is hypothesized that the structural properties of an osteochondral cylinder graft-recipient construct might be affected by the instability between the graft and the recipient before obtaining bony union. At postoperative weeks 3 and 8, newly formed tissue in the gap between the graft and the recipient and bone formation at the subchondral area were observed histologically suggesting the transplanted graft might be stabilized; however, the structural properties of the osteochondral cylinder graft-recipient construct were still low compared with that of the normal control model. Thereafter, at postoperative week 12, bone trabeculae decreased and was remodeled histologically and the structural properties of the osteochondral cylinder graft-recipient construct increased and recovered to the level of normal articular cartilage. In other words, the recovery of the structural properties of the osteochondral cylinder graft-recipient construct lagged behind that of the histologic findings. This finding is similar to the fracture healing process. [18-20] The cross-sectional area of a fracture site increases progressively until 4 weeks after the fracture. Thereafter, the cross-sectional area gradually decreases. The stiffness and peak torque to failure increases with fracture remodeling, however, until 8 weeks after fracture. The recovery of the mechanical properties after fracture lags behind the recovery of the morphology after fracture.

The tactile sensor used for measuring stiffness of articular cartilage in the present study is useful for determining stiffness of the area where the sensor touches, like the human hand.[11] The tactile sensor system was developed in medicine and robotics to detect the stiffness or softness of an object.[12,13,21-25] Uchio et al. assessed human cartilage stiffness of the femoral condyle and the patella with the tactile sensor system during arthroscopy and reported that the sensor system was useful for determining intraoperative stiffness of healthy or diseased human articular cartilage.[12] The sensor monitors stiffness at the site that the sensor probe touches. The stiffness of articular cartilage measured with this system might be affected by subchondral bone and evaluate the structural properties of the osteochondral cylinder graft-recipient construct. Therefore, it is possible that the sequential changes in the articular cartilage stiffness of the transplanted graft were affected by the remodeling process of the subchondral bone of the graft.

The weakness of the present study is that the mechanical and histological properties after the osteochondral transplantation were analyzed using a rabbit model. In human, the sequential changes of the stiffness of articular cartilage might be affected by lack of use after surgery or by rehabilitation.

CONCLUSIONS

In the present study using an animal model, the stiffness of articular cartilage of the osteochondral graft was normal at the time the graft was initially placed. At postoperative weeks 1, 3, and 8, the stiffness was lower than the normal cartilage stiffness and increase of bone trabeculae at sbuchondral area are histologically observed. At postoperative week 12, the stiffness of articular cartilage of the osteochondral graft was normal and bone traveculae at subchondral area is remodeled well. Athough the weakness of the present study is the analysis using a rabbit model, these changes of the structural properties of the osteochondral cylinder graft-recipient construct after the osteochondral graft surgery should be considered when making the rehabilitation program after surgery.

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LEGENDS

Figure 1: Tactile sensor system.

A tactile sensor probe with a 2-mm diameter hemispheric tip is placed on the surface of the articular cartilage. Overall view (A) and magnified view (B).

Figure 2: The stiffness of the articular cartilage.

Data measured with the sensor system are calibrated by the following calibration formula: stiffness (N/m^2) = 233.2 x Δf (Hz) +104948.0 (where R^2=0.91).

The average stiffness of articular cartilage of the transplanted graft at postoperative weeks 1, 3, and 8 is significantly lower than the normal cartilage stiffness. The average stiffness of articular cartilage of the transplanted graft, immediately after surgery and at potoperative week 12 is not significantly different from the normal cartilage stiffness.

*Statistically significant differences ($p < 0.05$) in unpaired t-tests between the stiffness of normal articular cartilage during different postoperative periods are shown.

Figure 3: Histologic findings stained with hematoxylin and eosin,immediately after surgery (A), at postoperative weeks 1 (B), 3 (C), 8 (D), 12 (E), and in the normal (F).

The Gap in the articular cartilage surface and discontinuity of bone trabeculae at the subchondral bone area is observed between the graft and the recipient, immediately after surgery (A). The gap in the articular cartilage surface between the graft and the recipient is observed and bone trabeculae at the border between the graft and the recipient increase, at postoperative week 1 (B). Continuity of the articular cartilage surface between the graft and the recipient and increase of bone trabeculae at sbuchondral area are observed at postoperative week 3 (C) and 8 (D). Continuity of the articular cartilage surface is improved and bone traveculae at subchondral area decrease at postoperative week 12 (E) which is almost the same as normal (F).

Arrows indicate the margins of the graft (original magnification, x 30).

Figure 1

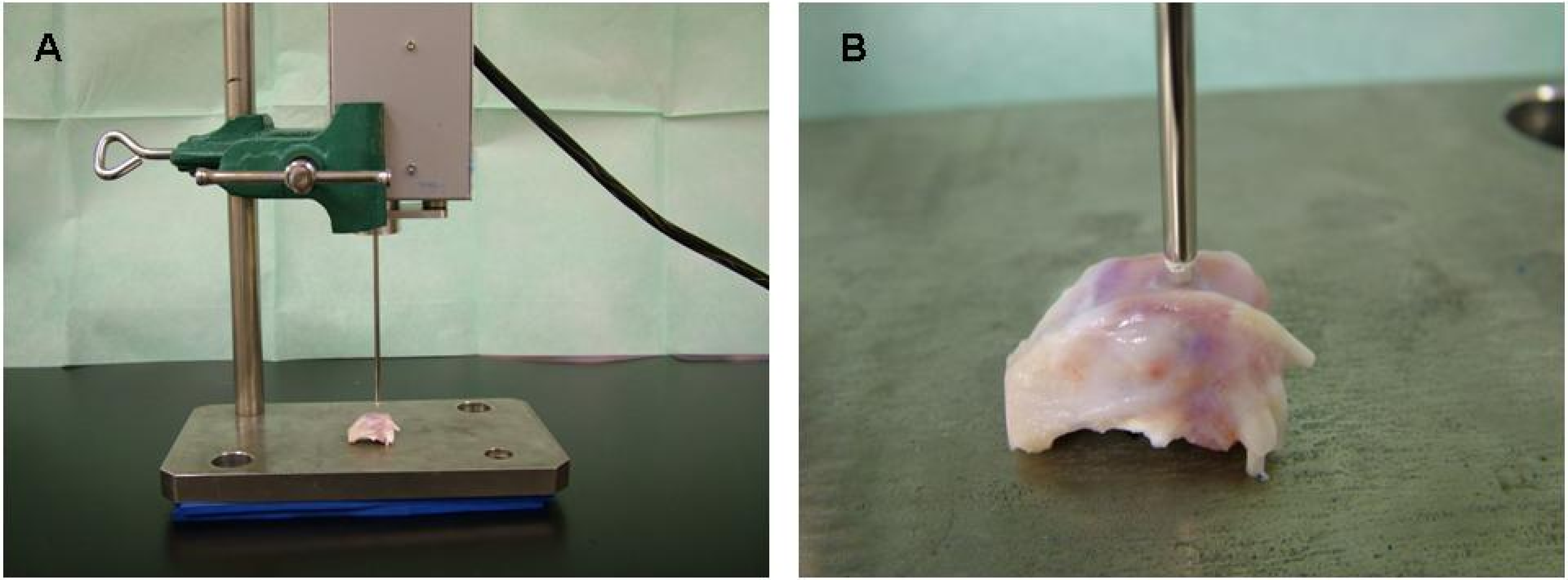
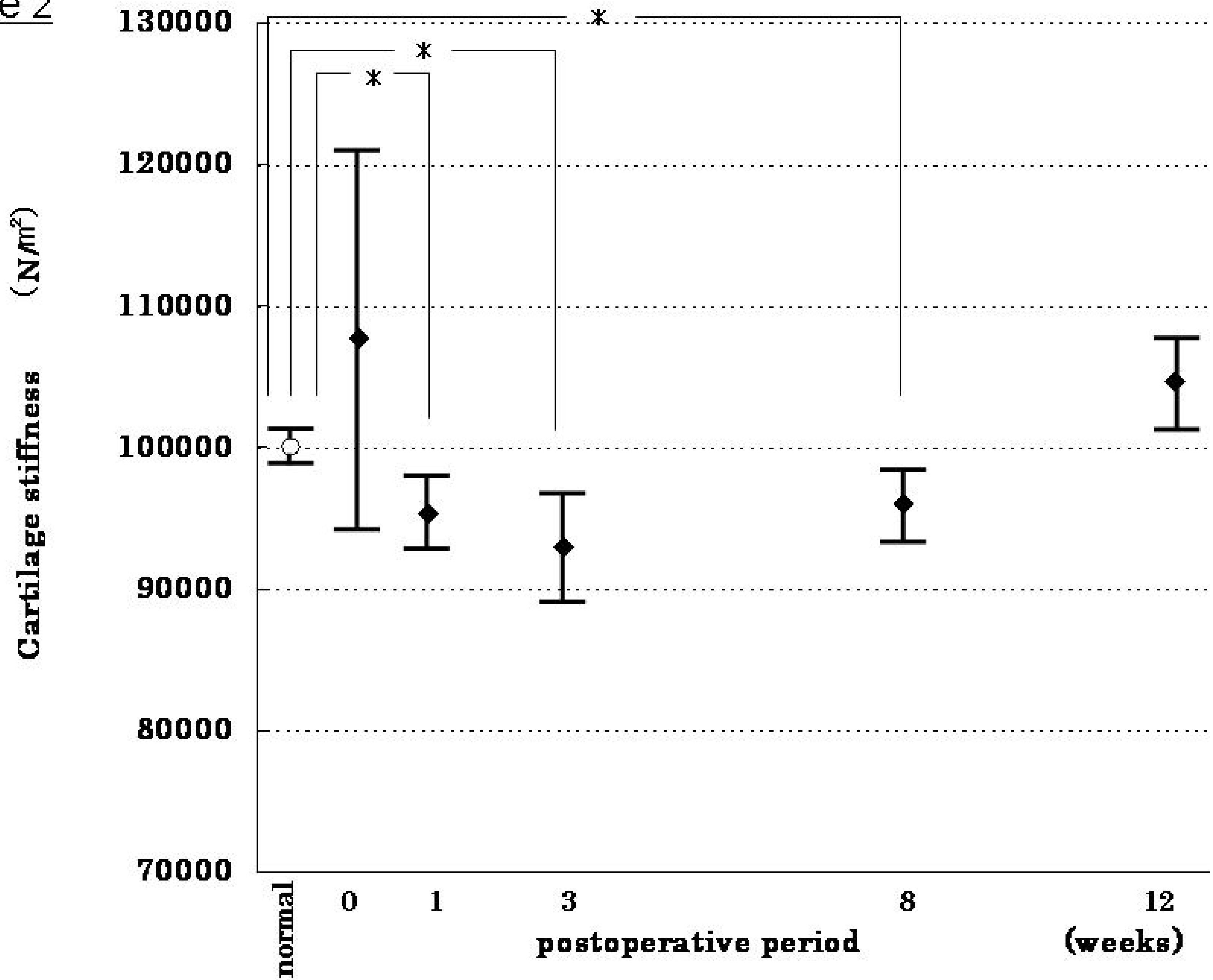


Figure 2



130000
120000
110000
100000
90000
80000
70000
Cartilage stiffness (N/m²)
*
*
*
normal
0
1
3
8
12
postoperative period
(weeks)

Figure 3