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Repairing Osteochondral Defects of Critical Size Using Multiple Costal Grafts: An Experimental Study

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Abstract

Objective: To investigate the feasibility of repairing osteochondral defects of critical size by performing mosaicplasty using multiple sliced costal cartilage grafts, which enables repair of extensively injured knees using grafts from a single rib. *Design*: Critical osteochondral defects were prepared on the femoral groove of skeletally mature Japanese white rabbits. Costal cartilage grafts from a single rib were harvested and sliced into multiple segments (approximately 3-5 mm in length). The defects were left untreated or repaired by performing mosaicplasty using costal cartilage grafts (with or without a longitudinal cut along the middle). At 4 and 12 weeks after transplantation, International Cartilage Repair Society macroscopic and histological grading was performed. *Results*: The macroscopic score and visual histological score were significantly higher in the repaired groups than in the untreated group at 4 and 12 weeks after surgery. Histological continuous integration between grafted costal cartilage and host bone was observed in both repaired groups. *Conclusions*: The findings suggest that costal cartilage might be a useful alternative source for chondral grafting. We were able to repair large osteochondral defects by performing mosaicplasty using multiple sliced costal cartilage grafts from a single rib.

Keywords

articular cartilage, costal graft, osteochondral defect, rabbit, mosaicplasty

Introduction

Autologous osteochondral grafting has been widely considered to be a reliable one-stage method for the treatment of damaged articular cartilage.[1] It has been confirmed that the cartilage in osteochondral grafts can maintain its biochemical and biomechanical characteristics after transplantation.[2] Although the clinical results from short-term follow-up studies are satisfactory, donor-site pain or morbidity in the injured knee is a concern with this technique. In addition, it is difficult to restore the contour of the femoral condyle using the grafts that cannot be further modified once collected.

Costal cartilage is hyaline cartilage and shows phenotypic similarities to articular cartilage[3]; hence, it can be another source for autologous graft in the reconstruction of articular cartilage,[4-6] external ears,[7] and the trachea.[8] The costal osteochondral junction has been employed to treat osteochondral impair not only in small joints such as the interphalangeal joint[9] and temporomandibular joints[10] but also in the elbows[11,12] and wrists.[13] The clinical results were satisfactory. However, the pleura anatomically attaches to the inner side of the rib closely around the costal osteochondral junction, which increases the risk of pleural injury during isolation.[14] Moreover, each rib has a single osteochondral junction; hence, it would be very invasive to repair large osteochondral defects when several costal osteochondral junctions are needed, which would cause a higher incidence of donor-site morbidity.[15,16]

We speculated that if the costal cartilage could biologically integrate with the underlying host bone, it would be possible to use multiple sliced costal cartilage grafts to repair extensive osteochondral defects in a manner similar to mosaicplasty. Thus, a knee joint (or elbow) could be restored using a single rib. In this study, we tested this

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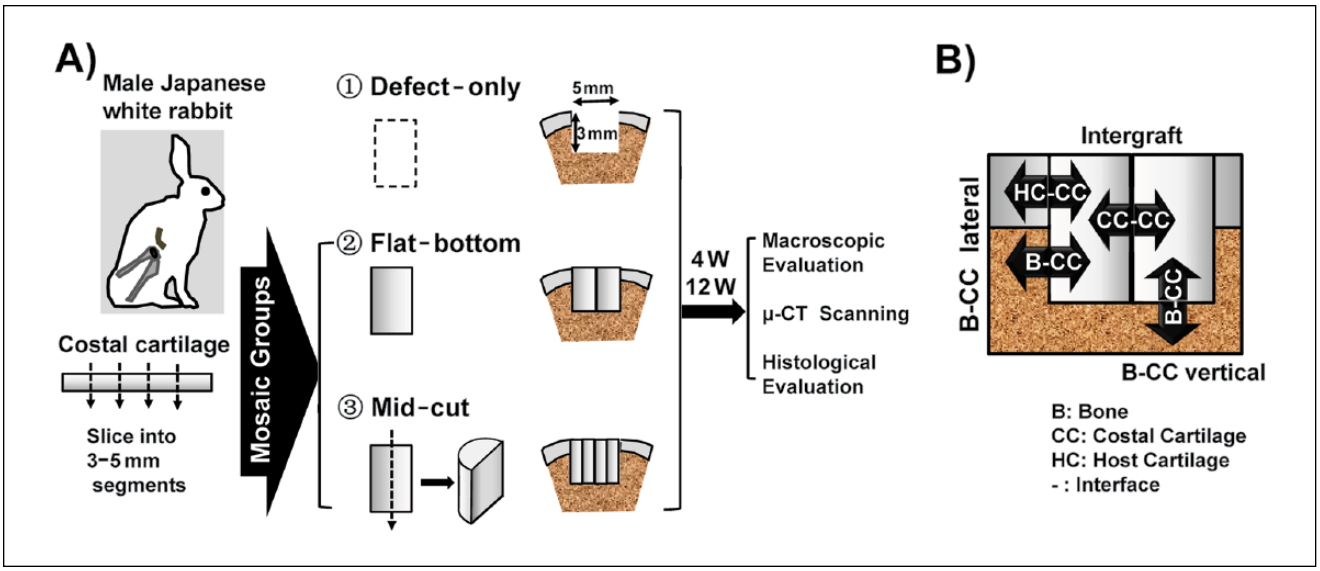

Figure 1. Illustration of the experimental design: (**A**) Preparation and implantation of the costal cartilage graft; (**B**) Illustration of histological interfaces.

proposal by evaluating the contour of repaired defects and the integration of transplanted costal cartilage and host bone in a rabbit model.

Methods

Operative Procedure

The approval for the animal study was granted by the animal laboratory of our institution. All procedures of this study followed the principles of the Declaration of Helsinki. Male Japanese white rabbits (20 weeks of age) regarded as skeletally mature young adults were used in this study. After anesthesia, an incision was made along anterior portion of the sixth or seventh rib on the right side. With careful protection of pleura, a 2.5-cm segment of costal cartilage was harvested from the sixth or seventh rib. The cartilage segment was sliced into approximately 3- to 5-mm segments after rinsing. The following procedures were used to repair the segments depending on the type of repair required for each knee (**Fig. 1A**): (1) flat-bottom, the segments above were used as direct grafts (14 knees), and (2) mid-cut, the grafts were further cut into half longitudinally (14 knees). The patella was dislocated laterally after a medial parapatellar approach. Articular osteochondral defects (5 mm in diameter and 3 mm deep) were prepared in the femoral groove of both knees using a trephine bur. The defects were either kept untreated (14 knees) or repaired by press-fit with each kind of prepared costal cartilage grafts in a mosaic fashion. Excess costal cartilage of the grafts protruding from the adjacent native cartilage was then shaved off to fit the defects using a scalpel. No immobilization was applied after surgery.

Macroscopic Evaluation of Cartilage Repair

The rabbits were euthanized under anesthesia at the end of the 4th and 12th weeks after surgery. Defect sites were observed macroscopically and assessed using the International Cartilage Repair Society (ICRS) macroscopic score according to the evaluation protocol.[17]

Histological and Immunohistochemical Processing

After macroscopic assessment of the cartilage repair, the distal femur was removed at the epicondylar level and fixed in 10% neutral buffered formalin. The samples were decalcified and embedded in paraffin. Four-micrometer sections were prepared sagittally along the midline of the osteochondral defect. Staining with hematoxylin and eosin (H&E), safranin O, and toluidine blue was performed in sections from each defect.

After blocking and antigen retrieval, all sections for immunohistochemistry were treated with mouse type I collagen and type II collagen monoclonal antibodies (Daiichi Fine Chemical Co. Ltd., Takaoka, Japan) diluted at 1:400 and 1:100, respectively, overnight at 4°C. After three washes, a peroxidase-conjugated secondary antibody was placed on the sections for 30 minutes. After washing, the sections were allowed to react with the substrate buffer and high-sensitivity substrate-chromogen (DAKO Corp., Carpinteria, CA) for 1 minute.

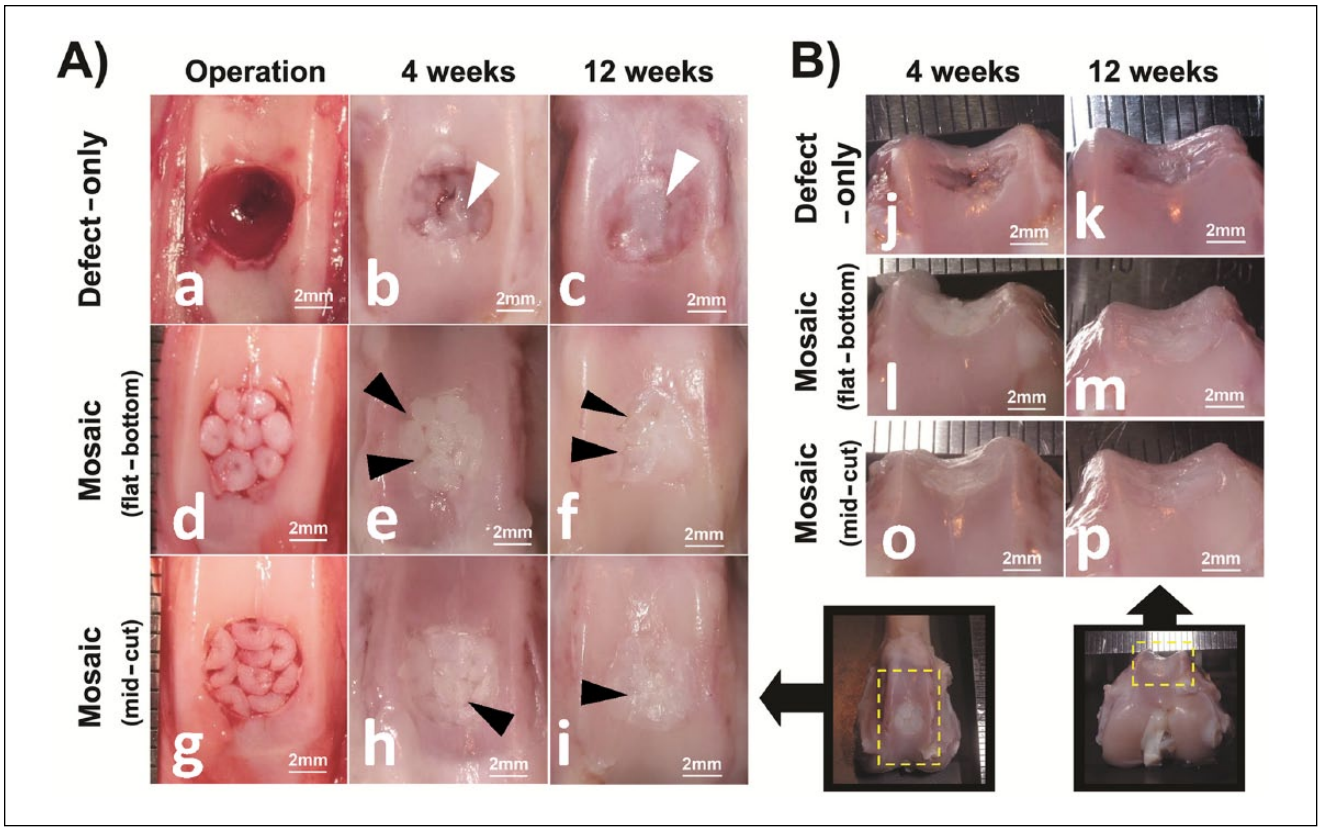

Figure 2. Macrophotographs of operated knees at the indicated periods after surgery treated with flat-bottom and mid-cut costal cartilage grafts or without treatment. (**A**) Anteroposterior view and (**B**) Tangential view. The white arrow heads indicate the soft tissue filled inside the defects without treatment; the black arrow heads indicate the intergraft or graft-host margins.

The collagen organization was evaluated using a Picrosirius Red Stain Kit (Polysciences, Inc., Warrington, PA) according to the manufacturer's instructions. A polarized light microscope (Olympus AX80; Olympus Optical Co. Ltd., Tokyo, Japan) was used to observe the results.

Histological Grading Score

The histology of repaired tissue at 4 weeks and 12 weeks was evaluated blindly using the modified histological grading scale as described by Wakitani (Please see the supplementary materials).[18,19] A new criteria category "Integration of Graft With Host Bone" was implemented in addition to the categories associated with the vertical integration of cartilage to the host bone.

Statistical Analysis

Descriptive statistics were used to determine group means and standard deviations for numerical data, and analysis was performed using analysis of variance. Statistical significance was assumed for P values of <0.05.

Results

Macroscopic Evaluation and Quantitative Scores

The operated knees showed no evidence of infection throughout the postoperative period.

At 4 weeks after surgery, the grafted costal cartilage of both mosaic groups was attached firmly to the bottom, and none was lost. As shown in **Figure 2Ae** and **h**, graft surfaces were white, smooth, and shiny like native articular cartilage. The borders of the grafts remained readily visible (**Fig. 2Ae** and **h**, black arrow head), which suggested the insufficient tissue integration between the grafts and peripheral articular cartilage, especially the intergraft region. The integration showed no obvious difference between the cut and intact sides of the grafts in the mid-cut group. Irregularly concaved surface was observed in the defect-only group (**Fig. 2Ab**, white arrow head). As shown in **Figure 2Bi** and **o**, the femoral patellar groove was well restored in both mosaic groups, which was confirmed from a tangential view; in contrast, the surface of the defect-only group was depressed irregularly (**Fig. 2Bj**).

At 12 weeks after surgery, graft surfaces were white and glistening. The borders between the grafts and peripheral

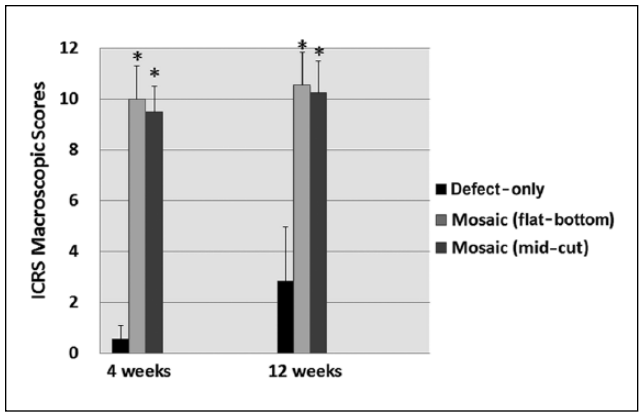

Figure 3. ICRS macroscopic scores for both mosaic groups (multiple costal grafts with either flat-bottom or mid-cut) and the untreated defect-only group after 4 and 12 weeks. * indicates significant difference between the mosaic group and blank group ($P < 0.05$; $n = 7$).

articular cartilage were not as clear as the ones at 4 weeks but still distinguishable, especially the intergraft gaps (**Fig. 2Af** and **i**, black arrows). In the defect-only group, the surface of the defect was still irregularly depressed with loose soft tissue covered, but smoother and more shallow (**Fig. 2Ac**, white arrow head) compared with the features at 4 weeks. The well-restored femoral patellar groove was still maintained in both mosaic groups according to tangential views, as shown in **Figure 2Bm** and **p**; in contrast, the defect surface in the control group was improved 8 weeks before but still irregular and concave (**Fig. 2Bk**).

At either 4 or 12 weeks after surgery, ICRS macroscopic scores were significantly higher in both mosaic groups than in the defect-only group ($P < 0.05$), but no significant difference was observed between both mosaic groups (**Fig. 3**). The scores were higher at 12 weeks than at 4 weeks in all three groups, but the differences were only significant in the defect-only group (**Fig. 3**).

Histological Evaluation and Quantitative Scores

At 4 weeks after surgery, there were no signs of inflammation or necrosis. The surface was depressed in the defect-only group, and there was fibrous-like tissue filling the defect (**Fig. 4a**); in contrast, the contour of the femoral groove was restored by the costal cartilage grafts in the mosaic groups (**Fig. 4e** and **i**). The cartilage matrix of the grafts were stained red by safranin O (**Fig. 4f** and **j**) and blue by toluidine blue (**Fig. 4c** and **g**), and some gaps were clearly distinguishable in the intergraft space and between the graft and peripheral host cartilage. The transplanted costal grafts showed intensity of collagen type II comparable with that of native articular cartilage (**Fig. 4h** and **i**). In higher magnification views, as shown in **Figure 5a**, the surface of defect-only group was covered with irregular fibrous tissue; the costal graft surface was smooth. There was newly formed cartilage-like tissue presented on the peripheral region of the host cartilage in the control group (**Fig. 5b**); the margin between the transplanted costal cartilage and host articular cartilage was still visible (**Fig. 5f** and **j**). The host bone tissue integrated with the implanted costal cartilage tissue both on the lateral side and on the bottom histologically. There was immature chondrogenic tissue observed on the costal graft surfaces (**Fig. 5g**, **h**, **k**, and **l**, white arrows), which might indicate that endochondral ossification was involved in the osteochondral integration process. The cartilage tissue in the costal grafts retained its hyaline cartilage appearance and stained well with safranin O (**Fig. 5e** and **i**), but the interfacial region close to the host bone showed many vacant cavities, some of which were invaded by newly formed bone tissue (**Fig. 5k** and **l**, black arrow heads). At 4 weeks, the overall histological scores for the defect-only group, mosaic group (flat-bottom grafts), and mosaic group (mid-cut grafts) were 1.86, 14.25, and 15.50, respectively ($P < 0.05$ compared with control group; **Fig. 6A**) The histological scores of integration for the mosaic group (flat-bottom grafts) and mosaic group (mid-cut grafts) were 3.00 and 4.25, respectively ($P > 0.05$; **Fig. 6B**).

At 12 weeks postoperation, in the defect-only group, fibrous-like tissue presented in the defects and the surface appeared fibrillated. There was newly formed bone in the defects, which even reached the level similar to that of the surrounding subchondral bone (**Fig. 4i**, black arrow head), but there was still no cartilage covering. The mosaic costal grafts maintained the contour of the femoral groove without collapse and necrosis (**Fig. 4q** and **u**). In higher magnification views as shown in **Figure 5**, the surface of the transplanted costal grafts still remained smooth without degeneration (**Fig. 5q** and **u**), in opposition to the irregular surface of the fibrous tissue in the defect-only group (**Fig. 5m**). There was cartilage-like tissue formed between the gap of the costal grafts and host cartilage with continuous toluidine blue staining (**Fig. 5r** and **v**). The lacunae close to the host bone were absent of cells inside, and some of the outermost ones were open and filled with bone matrix, which resembled an interlocking-like structure (**Fig. 5s**, **t**, **w**, and **x**, black arrow heads). Histologically, the newly formed interface between the costal cartilage and host bone was continuously integrated without gap and interstitial fibrous tissue both on the lateral and bottom osteochondral interface. There were still remnants of endochondral ossification, but the frequency was much lower at 12 weeks than at 4 weeks, and the tissue seemed more calcified (**Fig. 5w**, white arrow heads). At 12 weeks, the overall histological scores for the defect-only group, mosaic group (flat-bottom grafts), and mosaic group (mid-cut grafts) were 2.00, 15.25, and 14.50, respectively ($P < 0.05$ compared with

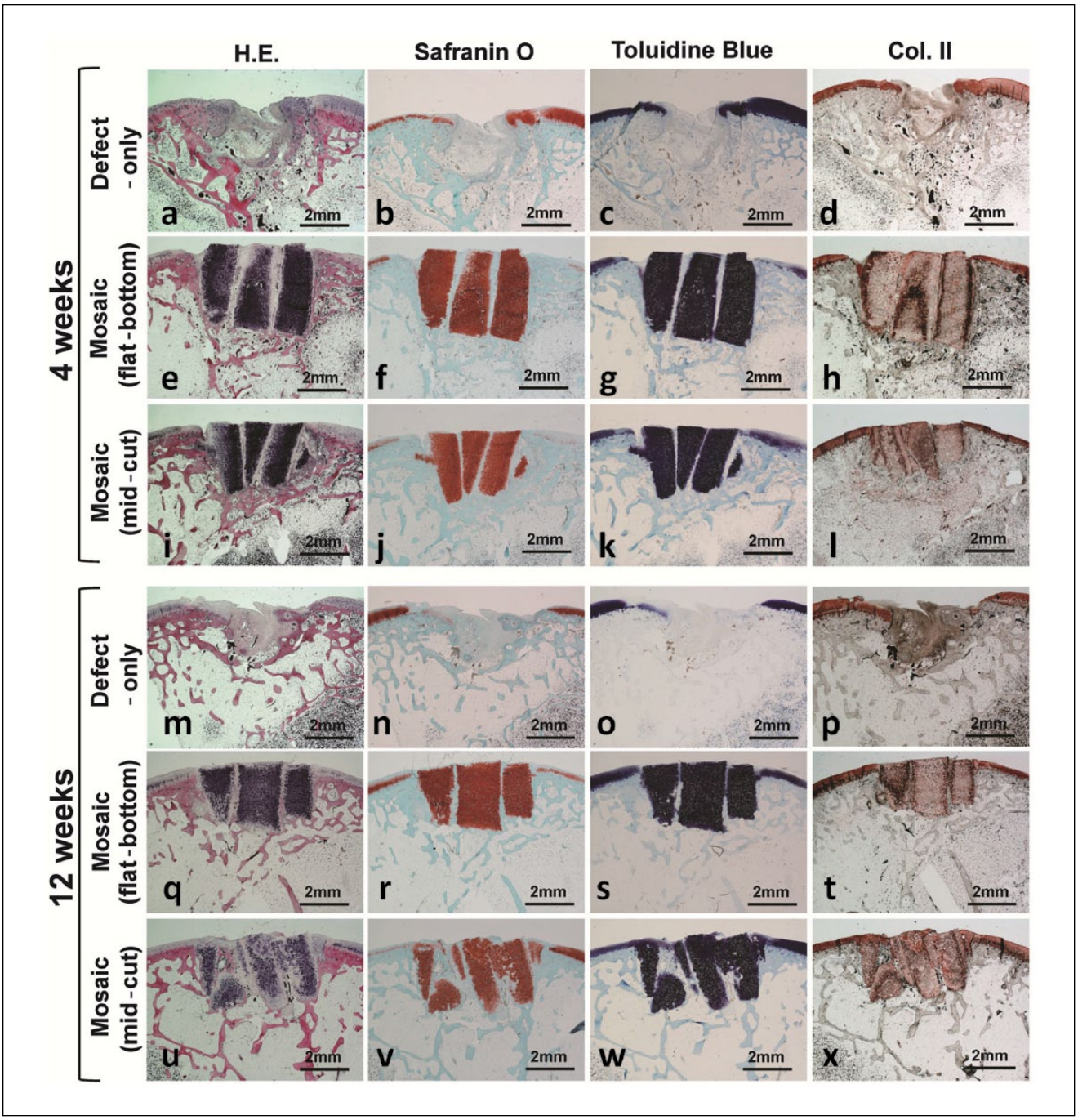

Figure 4. Histological findings for 4 (**a**-**i**) and 12 weeks (**m**-**x**) after surgery (scale bar = 2 mm, 1× objective). **a**, **e**, **i**, **m**, **q**, and **u** are hematoxylin–eosin staining images; **b**, **f**, **j**, **n**, **r**, and **v** are safranin O staining images; **c**, **g**, **k**, **o**, and **s** are toluidine blue staining images; **d**, **h**, **l**, **p**, **t**, and **x** are immunohistochemical staining images of type II collagen.

the defect-only group; **Fig. 6A**). The histological scores of integration for the mosaic group (flat-bottom grafts) and mosaic group (mid-cut grafts) were 4.25 and 4.75, respectively ($P > 0.05$; **Fig. 6B**).

The histological changes around the intergraft gap after 12 weeks of surgery are shown in **Figure 7**. In the upper and middle regions of the gap between the costal grafts without mid-cut, there was only fibrous tissue filling inside (**Fig. 7A**), and small blood vessels were visible; in the lower intergraft space, there was new bone formed and invading into the opened lacunae on the surface of the costal cartilage graft to form an interlocking-like structure (**Fig. 7C**, white

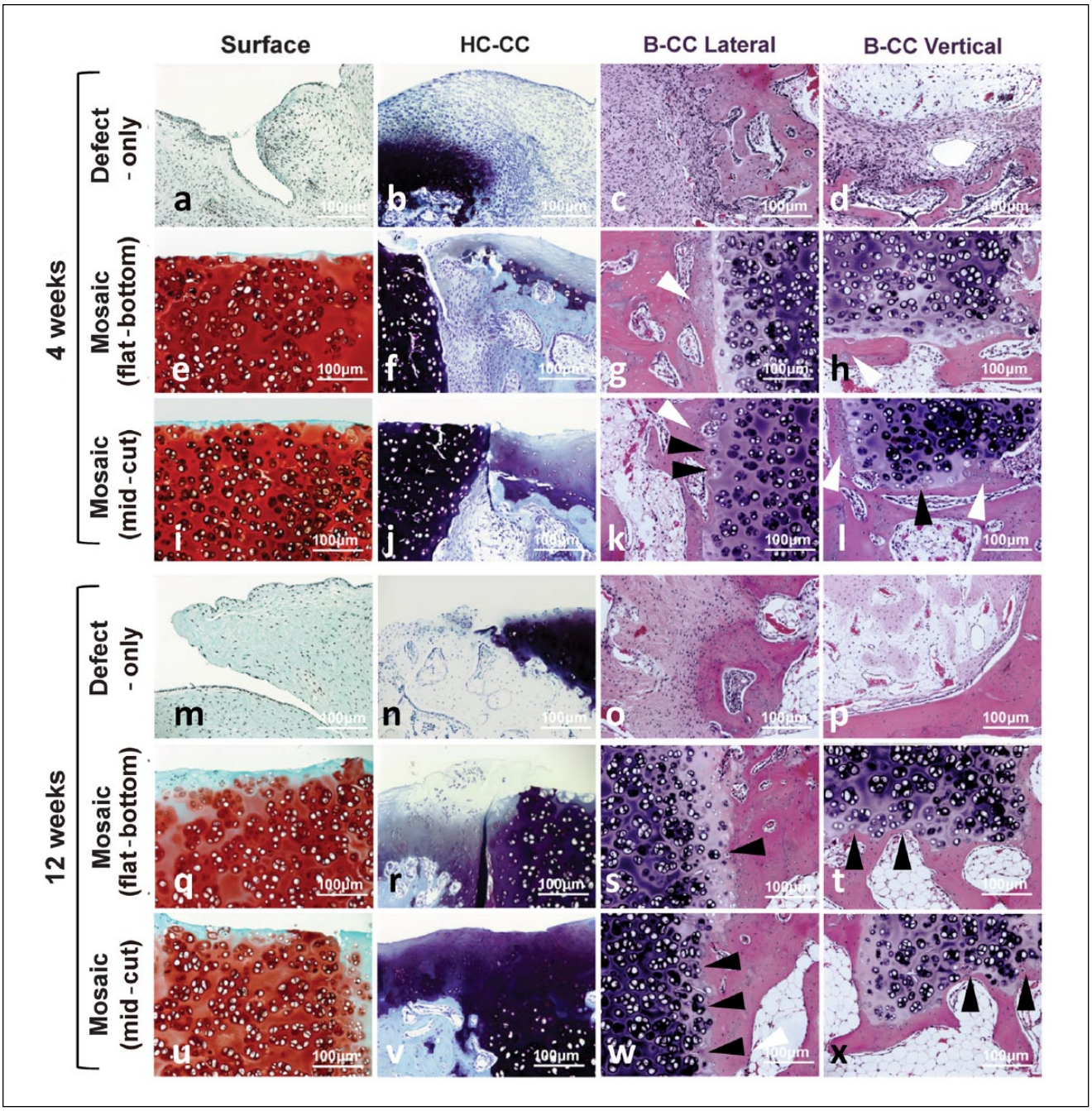

Figure 5. Histological findings of surfaces and integrations in the osteochondral defects at 4 (**a-i**) and 12 weeks (**m-x**) after surgery (scale bar = 100 μm, 10× objective). Shown in **a**, **e**, **i**, **m**, **q**, and **u** are safranin O staining images showing the surface of the defects; **b**, **f**, **j**, **n**, **r**, and **v** are toluidine blue staining images showing the margins of the defects and the integration of the peripheral host cartilage and grafted costal cartilage (HC-CC); **c**, **g**, **k**, **o**, **s**, and **w** are hematoxylin–eosin staining images showing the lateral interface between the host bone and grafted costal cartilage (B-CC lateral); **d**, **h**, **l**, **p**, **t**, and **x** are hematoxylin–eosin staining images showing the vertical interface between the host bone and grafted costal cartilage (B-CC vertical). The white arrow heads indicate chondrocyte-like cells in the B-CC interface, which suggests that endochondral ossification is involved in the integration process. The black arrow heads indicate the locations where bone tissue has invaded into the vacant lacunae of the costal cartilage and formed an interlocking structure.

arrows). This indicated that the costal cartilage had grafted into the internal region of the defect, which lacked contact with the peripheral host bone laterally and could be effectively anchored in the bone bed by the osteochondral

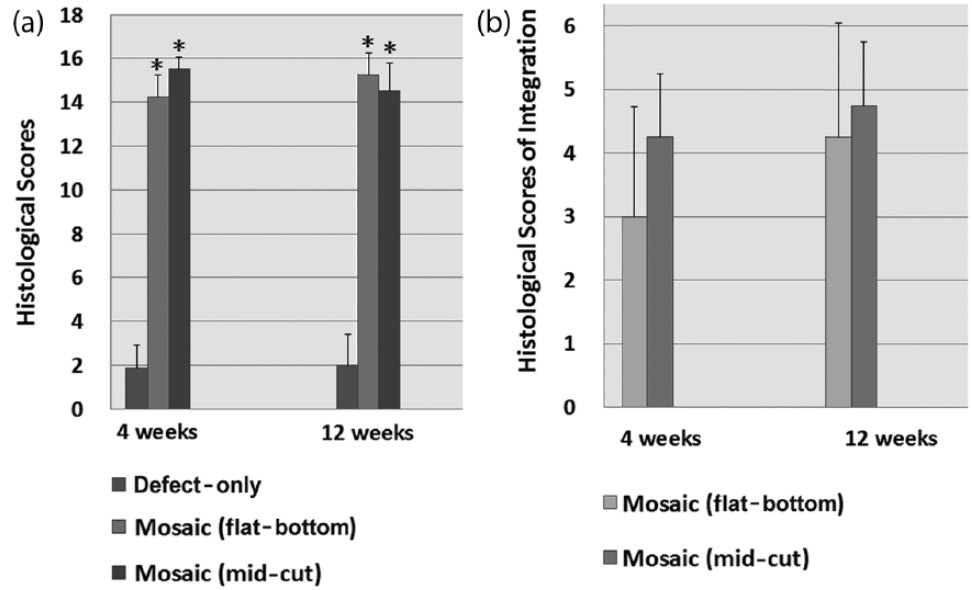

Figure 6. The histological scores for 4 and 12 weeks: (**A**) Overall histological scores; (**B**) Histological scores of integration. * indicates significant difference between the mosaic group and blank group ($P < 0.05$; $n = 7$).

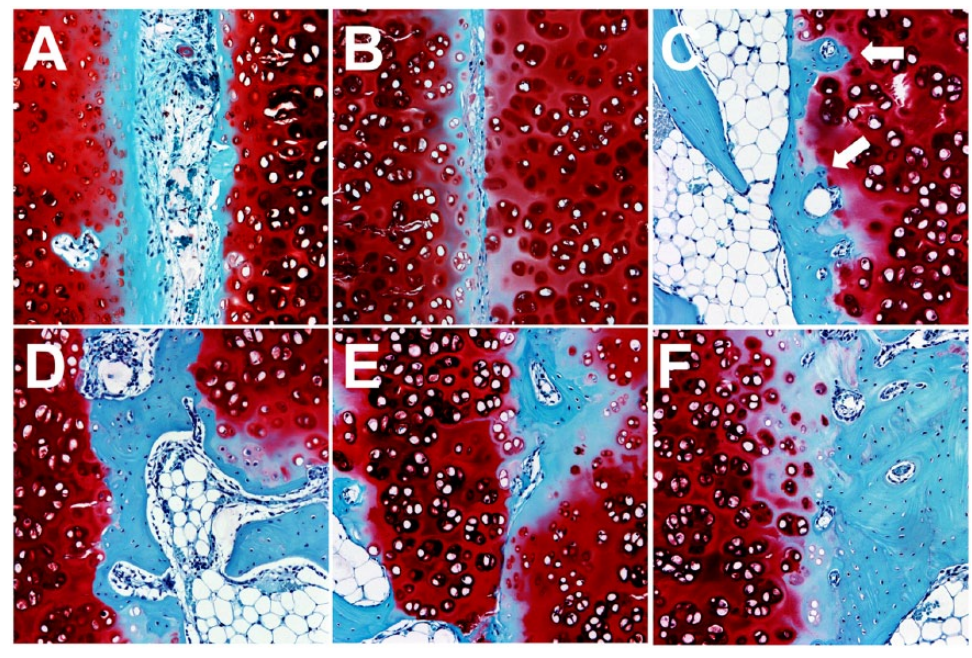

Figure 7. Histological findings around the intergraft gap after 12 weeks of surgery: (**A**)-(**C**) Mosaic group using costal cartilage without mid-cut (flat-bottom group); (**D**)-(**F**) Mosaic group using costal cartilage with mid-cut. (**A**) and (**D**) show the upper level; (**B**) and (**E**) show the middle level; (**C**) and (**F**) show the lower level. The white arrows indicate the interlocking-like structure.

integration mechanism. The in-between bone ingrowth from below was more significant in the group with mid-cut costal grafts, and the "bridge-like" bone formed even in the upper region of the intergraft space (**Fig. 7D**).

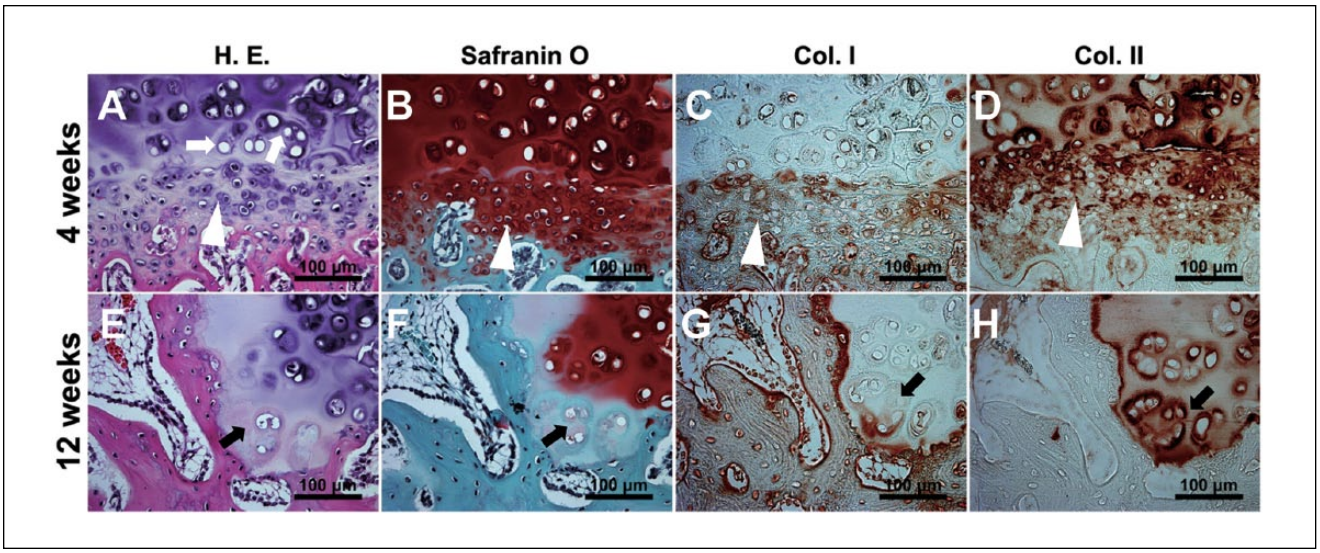

Figure 8. The newly forming osteochondral interface between the transplanted costal cartilage and host bone (flat-bottom mosaic group) at 4 weeks (**A**-**D**) and 12 weeks (**E**-**H**) after surgery. The white arrow heads indicate the chondrocytes filling in between the grafted costal cartilage and host bone bed; the white arrows indicate the lacunae close to the host bone, which were either vacant or had a hypertrophic chondrocyte inside; the black arrows indicate the costal cartilage matrix close to the host bone.

The newly forming osteochondral interface between the transplanted costal cartilage and the host bone (flat-bottom mosaic group) is shown in **Figure 8**. At 4 weeks posttransplantation, there were chondrocytes filling in between the grafted costal cartilage and the host bone bed (**Fig. 8A**-**D**, white arrow heads). The pericellular matrix was stained red, as was the cartilage, by safranin O (**Fig. 8B**, white arrow head). Immunological staining revealed that both collagen type I (**Fig. 8C**) and the collagen type II (**Fig. 8D**) distributed in the newly formed matrix between the costal cartilage and host bone. The lacunae close to the host bone were either vacant or had a hypertrophic chondrocyte inside (**Fig. 8A**, white arrow). At 12 weeks posttransplantation, there was mature bone lined on the costal cartilage grafts, and few chondrocytes were seen. The staining of the costal cartilage matrix close to the host bone was much thinner at 12 weeks than at 4 weeks (**Fig. 8E** and **F**, black arrow), which showed color between the cartilage and mature bone but still presented type II collagen (**Fig. 8H**, black arrow). Some lacunae were opened and had been invaded by newly formed bone. Compared with the appearance at 4 weeks, this finding indicated that a process resembling endochondral ossification might have been involved in the biological binding mechanism between the transplanted costal cartilage and host bone.

Viewing under a polarized light microscope, the collagen fiber orientation on the surface of the transplanted costal cartilage was mainly parallel with the surface and perpendicular with the longitudinal axis of the rib (**Fig. 9A**). The chondrocyte lacunae were absent of collagen fiber inside. Fibrous tissues had filled in the gap between the grafts, and collagen fiber orientation was longitudinal along with the gap (**Fig. 9B**). There was woven bone lining the costal graft at 4 weeks after surgery (**Fig. 9C**), but the lamellar bone component increased in this region at 12 weeks after surgery (**Fig. 9D**). At either stage viewing under polarized light, the border between the cartilage and bone was still clearly distinguishable, although there was no fibrous tissue and separation in between.

Discussion

Many reports have shown that mosaicplasty is an effective approach to resurfacing an osteochondral defect,[20] but concerns have increasingly been raised concerning secondary injury at the donor site when autografts are used[2] and there is disease transmission risk for allografts.[21] Because costal cartilage is also hyaline cartilage with active chondrocytes,[22] it is possible for autologous costal cartilage to be used as an alternative to articular cartilage in mosaicplasty. In a rabbit model, Sato *et al*.[23] successfully repaired osteochondral defects using osteochondral grafts prepared from the costal osteochondral junction, and the costal chondrocytes remained viable 12 months after surgery.[23] However, the defects in that study were only 2.5 mm and not of a critical size. Moreover, because there is only one osteochondral junction portion in a single rib, collection of multiple junctions for mosaicplasty would be very invasive. In addition, using a rabbit model, Mori *et al*.[24] prepared osteochondral grafts by mounting cancellous bone onto costal cartilage for 1 month and then used the preconstructed osteochondral graft to repair the osteochondral defect. However, the

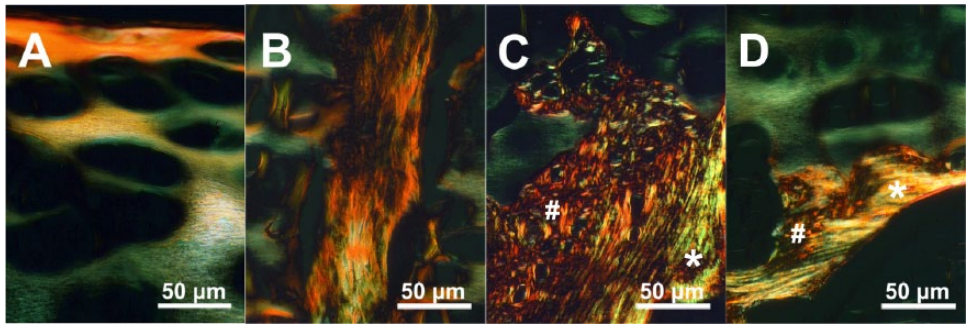

Figure 9. Polarized light microscopic findings: (**A**) The surface of the transplanted costal cartilage at 12 weeks after surgery; (**B**) The intergraft gap between the costal cartilage filled by fibrous tissue; (**C**) and (**D**) show the interface between the grafted costal cartilage and host bone at 4 and 12 weeks after surgery, respectively. # indicates the new formed woven bone in-between the grafted costal cartilage and host bone; * indicates the laminar bone.

patients had to undergo a second surgery, which would limit the clinical applications of this method. In this study, we successfully repaired a critically sized osteochondral defect by performing mosaicplasty using multiple sliced costal cartilage grafts collected from the costal cartilage portion, which showed that it was possible to repair critically sized osteochondral defects using sliced costal cartilage from a single rib without further injuring the impaired knee, as in traditional mosaicplasty.

Integration of the grafted costal cartilage with the surrounding bone bed is important for anchoring the transplanted hyaline cartilage portion and resurfacing of the osteochondral defect site. Our results (**Fig. 5**) showed that after 4 and 12 weeks of transplantation, the costal cartilage bound to the host bone, without fibrous tissue in between, not only vertically on the bottom but also laterally around the wall of the defect. At 4 weeks after surgery, histological staining revealed that there were chondrocyte-like cells in the host bone side close to the surface of the grafted costal cartilage, where not only type I but also type II collagen was present. The lacunae close to the host bone were either vacant or had a hypertrophic chondrocyte inside (**Figs. 5**, **7**, and **8**). At 12 weeks posttransplantation, most of the costal cartilage facing the host bone was lined with mature bone tissue. The costal cartilage matrix close to the host bone was less stained than it was 8 weeks earlier but type II collagen was still present. Some lacunae were opened and had been invaded by newly formed bone, which indicated that a process resembling endochondral ossification might be involved in the biological binding mechanism between the transplanted costal cartilage and host bone. After the surgery, the bone marrow from the trabecular bone bed filled to the gap between the grafted costal cartilage and host bone, together with bone marrow stromal cells that might initiate a bone-healing-like process on the surface of the cartilage. The bone marrow stromal cells differentiated into chondrocytes and then initiated a calcification process, which simulated endochondral ossification. Meanwhile, the chondrocytes in the lacunae close to the cartilage surface became hypertrophic or vacant, and the proteoglycans content was decreased in the corresponding region. Then, the lacunae opened, and the cavities were invaded by bone-forming cells so that the multiple bone structures protruding into the lacunae firmly locked the costal cartilage grafts to the host bone bed. This process may be similar to the healing process of the decreased and fixed cartilage fragments in osteochondritis dissecans patients.[25] However, the process observed has never been described previously.

Continuity and binding between the costal cartilage grafts with the surrounding host articular cartilage was not sufficient, which was consistent with the findings reported in many previous studies that used standard osteochondral grafts.[1] Because the chondrocytes in both the grafted and host cartilage were surrounded by a thick matrix, it would be difficult for them to migrate and "repair" the gap between them. Many efforts have been made to improve the binding between the graft and host cartilage, but the desired integration was still difficult to achieve.[23] The lack of continuity and binding was also observed between the costal cartilage grafts, especially in the upper level, although they were bonded to each other by newly formed bone tissue in the lower level and fibrous tissue in the middle level instead of cartilage tissue. The bone marrow stromal cells that came from the trabecular bone bed might have a role in this upward ossification in the intergraft gap. To improve the long-term outcome of costal cartilage transplantation, it might be useful to recruit and differentiate these cells into chondrocytes to improve the graft-host and intergraft integration.

In previous studies,[4-6,23] the costal cartilage grafts were prepared from the costal osteochondral junction. Because there is only one junction in a single rib, collection of

osteochondral junction grafts for resurfacing a large defect causes damage to multiple ribs, which would cause a higher incidence of chest deformities[15] and pleural injuries. Using the current approach, only one portion of costal cartilage may be sufficient for repair of a large-area defect, and the costal cartilage could be harvested through a small incision using a punch and the "moving window" technique. Moreover, because the pleura do not attach to the cartilage portion of the rib as close as the osteochondral junction portion and there is muscle tissue between them, the risk of pleural injury would be much lower by harvesting the grafts from the cartilage portion than from the junction portion. Because this one-stage approach also does not require cell processing and presents less risk of disease transmission than does an allogenic graft,[21] scarifying a portion of costal cartilage in one rib might be a good minimally invasive alternative to salvage one knee in patients.

The costal cartilage grafts were much thicker than were the surrounding articular grafts even after trimming. In this study, we proved the feasibility of creating conditions suitable for newly forming osteochondral interfaces between grafted costal cartilage and host bone. In future investigations, we would like to control the cartilage thickness of the costal grafts by drilling a hole to a specific depth on the bottom and filling trabecular bone inside to change the costal cartilage into osteochondral plugs. However, clinically, isolated costal cartilage grafts might not be thick enough to repair deep osteochondral defects. In this case, the defects might be repaired using osteochondral grafts fabricated by suturing the costal cartilage to a piece of trabecular bone, which could be collected from the bony portion of the rib minimal invasively by a trephine bur. Moreover, there are still some limitations in this study: as the high endogenous healing potential of the rabbit model makes translation to clinical biology difficult, large animal study is necessary to test the hypothesis using clinically relevant-sized costal chondral grafts; longer observation should be performed to investigate the changes in the costal cartilage posttransplantation.

Many regenerative methods have been investigated to restore injured articular cartilage, but they have not been perfected at present.[26] Mosaicplasty using autografts is still one of the most reliable approaches to restore large-area cartilage defects. The findings in this study suggest that costal cartilage might be a useful alternative source for grafting. In a mosaic manner, it is feasible to repair a large-area osteochondral defect using sliced costal cartilage grafts from a single rib.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

The approval for the animal study was granted by the animal laboratory of our institution. All procedures of this study followed the principles of the Declaration of Helsinki.

Supplementary Material

Supplementary material for this article is available on the Cartilage website at http://cart.sagepub.com/supplemental.

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