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# An enhanced method of rapid cartilage healing after costal cartilage resection

## Kostal kıkırdak rezeksiyonu sonrasında hızlı kıkırdak iyileşmesinin geliştirilmiş yöntemi

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***Background:*** In this study we aimed to compare the effects of leaving the perichondrial sheaths open, closing the sheaths reciprocally, and leaving the resected cartilages in the perichondrial bed as small autologous grafts to clarify the effect of perichondrium left intact within the donor site on costal cartilage regeneration.

***Methods:*** In this study, eight domestic pigs (Sus Scrofa Domestica) with a mean age of six months were used. Following resection of the sixth and seventh costal cartilages, costal cartilage beds were assigned to three groups. In group 1, perichondrial sheaths were left open, while muscles and other layers were closed. In group 2, perichondrial sheaths were reciprocally sutured with a soluble material. In group 3, grafts of 3 mm cubes, prepared from the resected costal cartilages of each subject, were located in the perichondrial beds and closed.

***Results:*** Costal cartilage regenerations in all three groups were scored and compared. Statistically significant differences were found among the groups (chi-square= 20.227, p<0.001). Group 1 had the lowest median score, compared to the other groups with a statistically significant difference. In group 3, the examination revealed that chondrocytes of newly developing fibrous cartilage were found to penetrate towards the cartilages located inside in seven of 20 preparations.

***Conclusion:*** This study shows that autologous cartilage pieces left in the perichondrial space accelerate cartilage regeneration and lead to spontaneous self-oriented chondrocyte migration, contributing to the stability of thoracic wall.

*Key words:* Autologous graft; costal cartilage; perichondrium.

***Amaç:*** Bu çalışmanın amacı, donör alanı içerisinde sağlam bırakılan perikondriyumun kostal kıkırdak rejenerasyonu üzerindeki etkilerini belirlemek için, katları karşılıklı kapatarak perikondriyal katları açık bırakmanın ve rezeke edilen kıkırdağı perikondriyal yatakta bırakmanın etkilerini karşılaştırmaktır.

***Çalışma planı:*** Çalışmada ortalama altı aylık olan sekiz evcil domuz (Sus Scrofa Domestica) kullanıldı. Altıncı ve yedinci kostal kıkırdakların rezeksiyonunu takiben, kostal kıkırdak yatakları üç gruba ayrıldı. Grup 1'de kaslar ve diğer katlar kapatılırken perikondrial yataklar açık bırakıldı. Grup 2'de perikondrial yataklar çözünür bir materyal ile kapatıldı. Grup 3'te ise rezeke edilen kostal kıkırdaklardan hazırlanan küp şeklinde 3 mm boyutlarında kostal kıkırdak parçaları perikondrial yatak içine konarak perikondrium kapatıldı.

***Bulgular:*** Üç grupta da kostal kıkırdak rejenerasyonu skorlandı ve karşılaştırıldı. Gruplar arasında istatistiksel olarak anlamlı farklılık olduğu saptandı (Ki-kare= 20.227, p<0.001). Grup 1 diğer gruplarla karşılaştırıldığında istatistiksel olarak anlamlı bir fark ile en düşük medyan skora sahipti. Grup 3'te yapılan incelemede 20 preparatın yedisinde yeni oluşmakta olan fibröz kıkırdağa ait kondrositlerin, perikondrial yatakta bırakılan kıkırdak küplerin içine doğru penetre olduğu görüldü.

***Sonuç:*** Bu çalışma perikondrial boşluğa bırakılan otolog kıkırdak parçalarının kıkırdak rejenerasyonunu hızlandırdığını ve toraks duvarı stabilitesine katkıda bulunarak, kendiliğinden gerçekleşen kondrosit yayılımına neden olduğunu göstermektedir.

*Anahtar sözcükler:* Otolog greft; kostal kıkırdak; perikondrium.

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Costal cartilage resection is performed by thoracic surgeons to correct thoracic deformities.[1] In addition, otorhinolaryngologists and plastic surgeons use costal cartilages to obtain autologous graft material. Deformities and thoracic wall instability of the donor site are significant complications, particularly following costal cartilage resection for aesthetic purposes.

Minimal cartilage resection has been suggested when resecting costal cartilage in order to produce faster healing.[2-4] It has also been shown that even after the resection of a limited number of costal cartilages, thoracic development deteriorates and the anteroposterior diameter of the thorax especially decreases.[2] Wherever possible, costal cartilages are harvested by both thoracic surgeons working on essential corrections in pectus surgery and otorhinolaryngologists and plastic surgeons who require them for graft material.

Leaving the perichondrium within the donor site is recommended in many surgical techniques in which cartilage resection is applied.[3-8] Subperichondrial resection of deformed costal cartilage was first defined by Ravitch[1] and has become the most preferred treatment method used in pectus deformities. It is known that cartilage regeneration occurs by the transformation of progenitor cells in the perichondrium into chondroblasts.[4] Baronofsky[6] and Welch[7] suggested that continuity of the perichondrium during resection of deformed cartilages is necessary for cartilage regeneration.

Recently, Genç et al.[3] and Kawanabe and Nagata[5] have reported that cartilage healing increases when a certain amount of resected costal cartilages are relocated within the perichondrial bed as little cubes.

In this study, we aimed to compare the effects of leaving the perichondrial sheaths open, closing the sheaths reciprocally, and leaving the resected cartilages in the perichondrial bed as small autologous grafts to clarify the effect of the perichondrium being left intact within the donor site on costal cartilage regeneration.

## MATERIALS AND METHODS

Eight Bama miniature pigs (Sus scrofa domestica) with a mean age of six months and weighing 65±5 kg were used. All animals received humane care in compliance with the European Convention on Animal Care and our institutional Ethics Committee approved the study. All subjects fasted for 12 hours before the operation. For premedication, atropine 0.05 mg/kg body weight was given intravenously to all subjects. A single dose of oxytetracycline 10 mg/kg body weight was injected intramuscularly before surgery for prophylaxis. Subjects were placed in a supine position on the operating table. Anesthesia was administered via intramuscular injection of 2 mg/kg body weight xylazine hydrochloride and 20 mg/kg body weight ketamine hydrochloride. After intravenous propofol administration (approximately 4-6 mg/kg body weight), which allowed for intubation, anesthesia was maintained with sevoflorane.

All subjects were operated on under sterile conditions with the same surgical procedure described below. The subjects' chests were shaved then prepared with a povidone-iodine solution. An incision was made from the lower end of the sternum and extended to the arcus costarum. The incision was carried through the subcutaneous tissue and rectus muscles. Then the sixth and seventh costal cartilages were identified. An incision was made via Bovie electrocautery on the perichondrium, and then the costal cartilages were freed from the perichondrium and removed.

To avoid the complications of possible paradoxical respiration due to the impaired stability of the thoracic wall, only two costal cartilages were resected for each subject. A total of 16 costal cartilages were removed. Each perichondrial space in each subject was allocated to a different group. Three groups were designed to investigate the role of evacuated perichondrial beds in healing. In the first group (group 1), perichondrial sheaths of five costal cartilages were left open while the muscles and other layers were closed. In the second group (group 2), perichondrial sheaths of five costal cartilage beds were reciprocally sutured with absorbable material. In the third group (group 3), grafts of 3 mm cartilage cubes were placed into the perichondrial space in the six costal cartilage beds which had been prepared from the resected costal cartilages of each subject and closed.

During the postoperative period, daily oxytetracycline 7.5 mg/kg body weight given once a day was continued for antibiotic therapy. For pain control, fentanyl transdermal patches were used postoperatively.

All subjects were reoperated on after a four-weeks. Costal cartilages were reached again through the previous incision sites, and newly formed costal cartilages along with perichondriums were resected. One specimen in group 3 was excluded from the study because of lysis in the costal cartilage due to infection.

From each of the dissected costal cartilages, four sample sections of 2 mm were prepared for microscopic examination; thus, there were 20 (5x4) samples for each group (the sample sections were taken at least 10 mm apart from each other). The samples were put into a 10%

**Table 1. Modified scoring system of Hou et al.[5] for the evaluation of cartilage regeneration.**

| Score | Histopathological scoring in cartilage regeneration |
|---|---|
| 1 | There is fibrous tissue totally |
| 2 | Fibrous tissue is dominant but there is little cartilage |
| 3 | There are fibrous tissue and cartilage in equal amounts |
| 4 | Cartilage development is dominant but there is little fibrous tissue |
| 5 | There is cartilage totally |

buffered formaldehyde solution and then were removed into a buffered formic acid solution for decalcification. The samples yielded 4 $\mu$m sections which were then embedded into paraffin after processing. The sections were stained with hematoxylin-eosin, which is used for routine histopathological evaluation. For evaluation of scar tissue and new cartilage development, a modification was made for this study to the digital histological scoring system that Huo et al.[9] used in callus development (Table 1). In group 3, the added cubic hyaline cartilages were not included in the scoring system. The same pathologist, who was blinded to the subject information, scored all preparations (Figures 1 and 2).

### Statistical analysis

Data was evaluated with the Shapiro-Wilk test for concordance to normal distribution. No accordance to normal distribution was found. Then, the Kruskal-Wallis test was applied in order to find the differences between the groups. To assess multiple comparisons, the Mann Whitney U-test with Bonferroni correction was used. The chosen alpha level was 0.05 as a level of significance, and for multivariate analyses, the level was 0.017 after dividing the level into the comparison number. For statistical analyses, Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA) version 15.0 software was used. The power of the study was 94% with a G*Power 3.0.8 program. For one-way analysis of variance (one-way ANOVA), the power was calculated, and the result was multiplied by 0.95. It was then corrected for Kruskal-Wallis using Pitman's approach to asymptotic relative efficiency.

## RESULTS

In group 3, one sample was excluded from the study due to lysis caused by infection in the costal cartilage. Sixty sections from 15 regenerated costal cartilages were examined. First, the contours of the newly developed

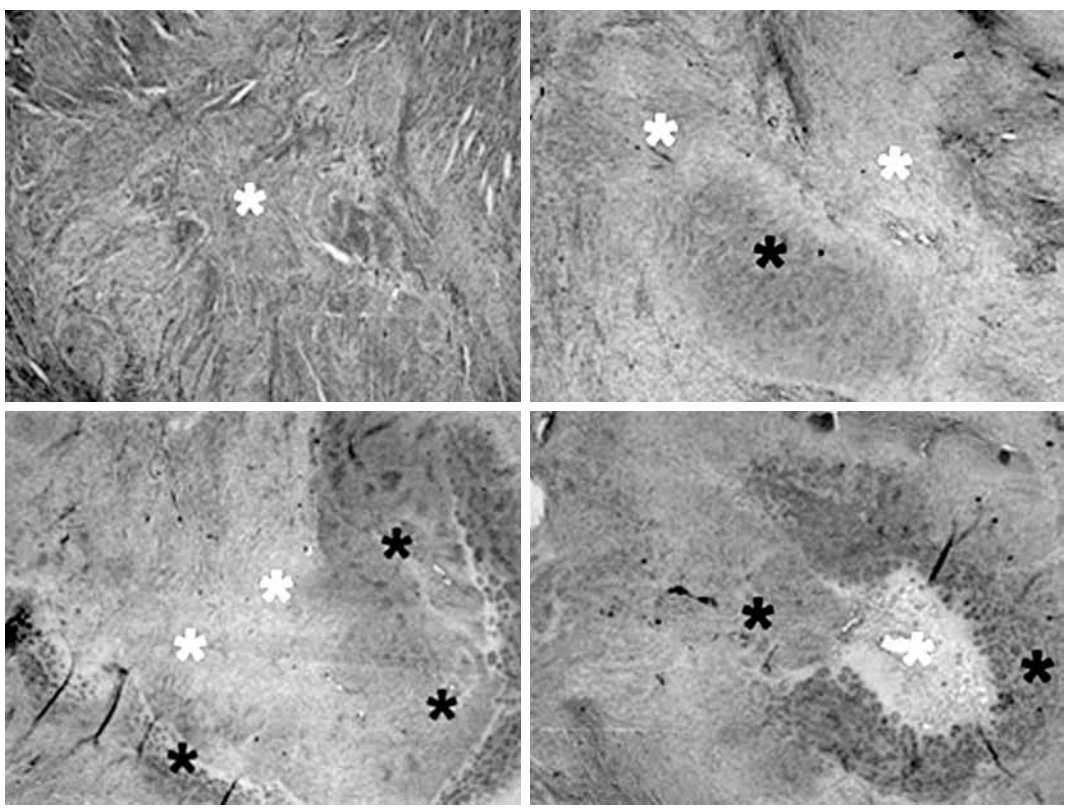

**Figure 1.** In the scoring system, views of costal cartilage regeneration score 1 on upper left, score 2 on upper right, score 3 on lower left and score 4 on lower right (white star indicates fibrotic areas, black star indicates neocartilagogenetic areas), (H-E x 50).

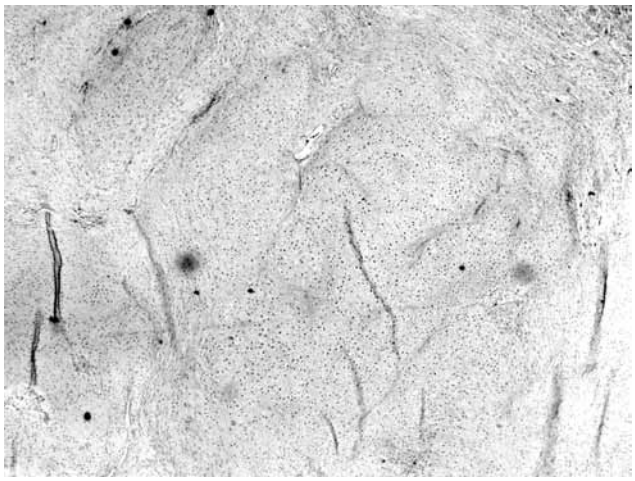

**Figure 2.** In the scoring system, histological view of score 5 which is formed by totally neocartilaginous tissue (H-E x 100).

costal cartilage were evaluated macroscopically. In group 1, 80% of the preparations' perichondrial contours were irregular. In the other two groups in which the perichondrium was closed, 80% of the preparations' perichondrial contours were regular (Figure 3).

Neovascularization was observed in the center of the regenerated cartilage in groups 2 and 3 in which the perichondrial sheaths were closed (Figure 4). However, in group 1, there were no signs of vascularization.

Costal cartilage regenerations in all three groups were scored and compared. In group 1, the mean cartilage regeneration score was 3.10 (median=3.10, minimum=2, maximum=4). In this group, cartilage development was peripheral and located close to the perichondrium while dense scar tissue was observed in the center. The mean cartilage regeneration score for group 2 was 3.70 (median=4, minimum=3, maximum=4). The perichondrium was closed in this group, and no cartilage particles were put into the perichondrial beds. However, the mean microscopic score was 4.15 (median=4, minimum=3, maximum=5) for group 3. The perichondrium was also closed in this group, but cartilage particles were put into the perichondrial beds (Table 2).

Test results showed statistically significant differences in the mean scores of all three groups (chi-square=20.227, $p<0.001$). To detect the source of the difference, the Mann Whitney U-test with Bonferroni correction was used, and the results are given in Table 3. Group 1 had the lowest the median score compared with the other groups, and according to the results of the Mann Whitney U-test with Bonferroni correction, this group was statistically different from the others.

In seven of 20 tissue sections obtained from the specimen of group 3, chondrocytes were determined to penetrate toward the cartilages located inside belonging to newly developed fibrous cartilage (Figure 5). Since it is impossible to calculate the ratio of cartilage graft within the section area related to the whole regenerated cartilage, no measurement was made of the percentage of penetration.

## DISCUSSION

Cartilage regeneration is maintained by a tentative balance between type 1 and 2 collagens. Scar tissue that occurs in an injury basically includes type 1 collagen while cartilage basically includes type 2 collagen. Because of the faster production rate of the type 1 collagen, it is important to increase cartilage regeneration during cartilage repair.[4] When the perichondrium remains unsutured, connection of the intraperichondrial space with injured muscle tissue causes an increase in type 1 collagen inside the perichondrium and disturbs the normal cartilage healing. Fonkalsrud reported that the closure of perichondrial sheaths by primary sutures provides better healing for the resected cartilage site; however,

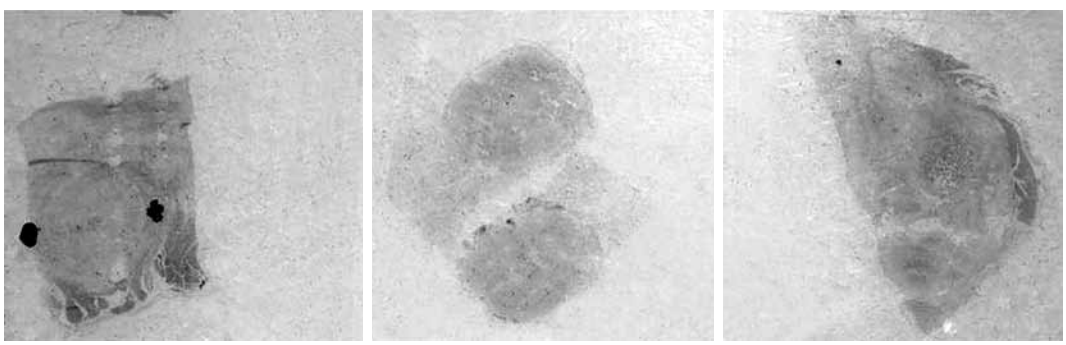

**Figure 3.** Macroscopic contour change in the regenerated cartilage. On the left, a sample for cartilage development with regular contours in group 3. The sample in the middle belongs to a normal costal cartilage. On the right, irregular cartilage development in a sample from group 1.

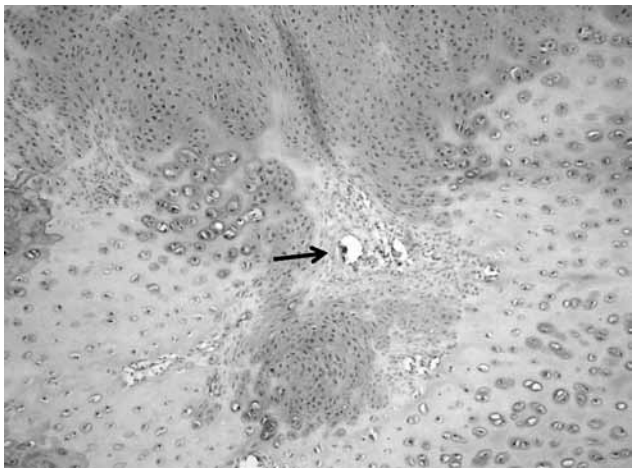

**Figure 4.** The newly developed fibrocartilaginous tissue on the upper part was observed to penetrate towards the lower hyaline cartilage. Vascularization in the center of the tissue was also noticed (H-E x 200).

**Table 2. Distribution of mean scores, medians, minimum and maximum scores of preparations according to groups**

| Groups | n | Mean±SD | Median | Min.-Max. |
|---|---|---|---|---|
| Group 1 | 20 | 3.10±0.72 | 3 | 2-4 |
| Group 2 | 20 | 3.70±0.47 | 4 | 3-4 |
| Group 3 | 20 | 4.15±0.59 | 4 | 3-5 |
| *Total* | 60 | 3.65±0.73 | 4 | 2-5 |

SD: Standard deviation; Min.: Minimum; Max.: Maximum.

**Table 3. Results obtained from comparisons of groups by Mann Whitney U-test after Bonferroni correction**

| Group (i) | Group (j) | Test statistics | *p* |
|---|---|---|---|
| I | II | 2.764 | 0.012* |
| I | III | 4.046 | 0.000* |
| II | III | 2.455 | 0.04 |

*: Significant after Bonferroni correction.

in the case of injury in the perichondrium, costal cartilage may heal with a calcified, rigid, and non-elastic bone in many patients. He concluded that this might lead to a rigid cylindrical thoracic wall.[8]

In our study, the perichondrium was kept intact at the donor site in all groups. Scoring of the regenerated cartilage in group 1 was detected as being lower than the other two groups due to the faster development of scar tissue regeneration in the center of the regenerated cartilage. However, it was clearly shown in this group that regenerated cartilage develops from the perichondrium and moves towards the center (Figure 3).

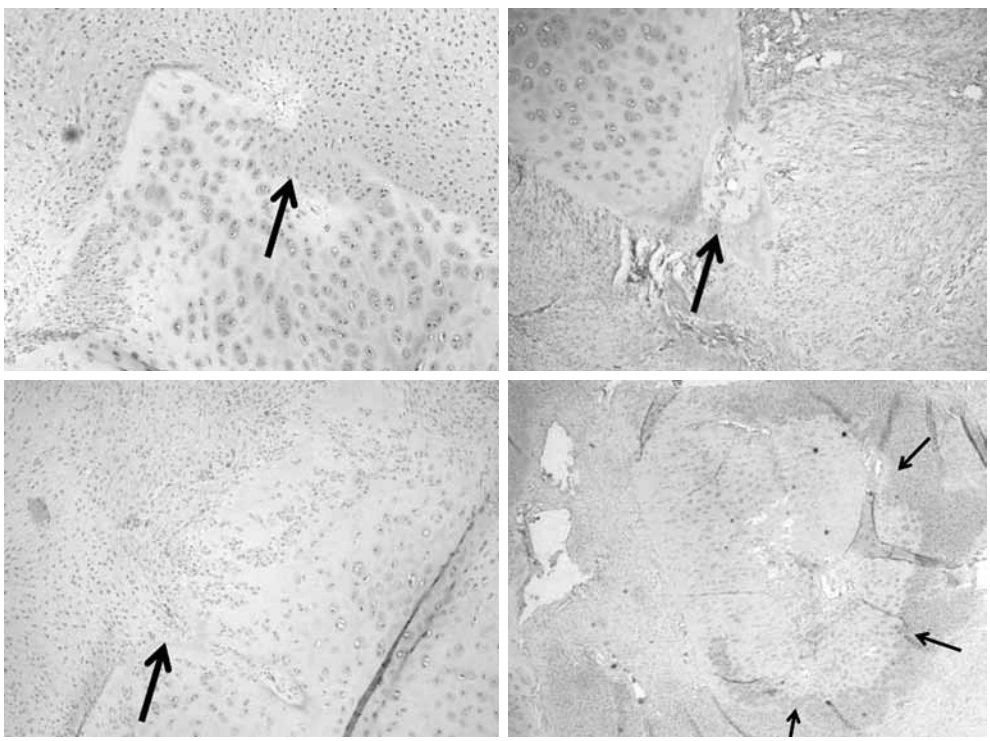

**Figure 5.** Penetration of fibrocartilage towards hyaline cartilage on the left lower-upper and right upper samples (thick arrow) (H-E x 100). On the right lower sample, it was observed that the cubic hyaline cartilage particle was penetrated from three different sides (thin arrows) and began melting (H-E x 50).

In the normal development of costal cartilage regeneration, the fibroblasts first differentiate and produce a cartilage matrix and/or fibrillary elements in the space in the perichondrium. The maturation of fibrocartilage to hyaline cartilage occurs and is known as oppositional growth. Neovascularization is observed in healthy developing fibrocartilage, and as the hyaline cartilage development proceeds, vascularization vanishes.[5] In our study, we detected neovascularization in sections of groups 2 and 3 (Figure 4). However, no neovascularization was detected in group 1 in which the perichondrial sheaths had been left open. This is thought to be because the scar tissue in the center led to the absence of normal cartilage regeneration.

The method of putting the resected cartilage back was first suggested by Genç et al.[3] in 2006 in corrective operations performed on thoracic wall deformities. Kawanabe and Nagata[5] suggested leaving cartilage particles in the perichondrial bed after cartilage resection to obtain an autologous graft. Researchers have proposed leaving small cubic grafts in the perichondral space and have reported how this contributes to cartilage development.[3,5] Investigation of regenerated cartilages at the sixth, eighth, tenth, and twelfth months has revealed that the autologous graft disappears in the regenerated cartilage via chondrocyte invasion.[5]

In group 3, in which 3 mm cubic autologous grafts were located in the perichondrial space, we found higher hyaline cartilage development scores than they were in the other two groups. We do not think this is because of the cartilage-developing effect of the grafts left in the perichondrial space but rather that it can be attributed to the decreased area of the perichondrial space and more chondrocyte accumulation around the grafts due to this decrease. Additionally, the penetration of the chondrocytes of the newly developed hyaline cartilage into the autologous graft works as a type of grout between the more mature autologous cartilage and the regenerated cartilage. This increases its resistance.

Researchers have investigated the effect of human amniotic fluid, growth-increasing factors like erythropoietin, and mechanical induction on costal cartilage regeneration.[10,11] It has been shown that all these factors have been contributors to this regeneration. However, no definitive result has been given in terms of utility in surgery.

We think that leaving a portion of autologous cartilage in the perichondrial space is an acceptable and simple approach for thoracic surgeons, otorhinolaryngologists, and plastic surgeons for the sake of time and effort. Our study showed that autologous cartilage particles left in the perichondrial space accelerated cartilage regeneration and contributed to maintaining stability by engendering self-oriented chondrocyte migration.

**Declaration of conflicting interests**

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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