



Doctor of Philosophy

Thin-solid block and diced cartilages: the best option for dorsal augmentation using costal cartilage (an experimental study in rabbits)

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ENGLISH ABSTRACT

Objectives. Costal cartilage is commonly used as a dorsal implant in Asian rhinoplasty. To achieve better outcomes, it is important to know which types of costal cartilage are most appropriate for dorsal augmentation. Therefore, this study aimed to investigate how various forms of costal cartilage affect the surrounding tissues and their resorption over time, as well as their clinical appearance, using histomorphological analysis.

Methods. Cartilage samples were collected from the anterior chest wall of 10 rabbits. Four forms of cartilage, i.e., 2-mm solid block, 1-mm solid block, diced, and crushed, were prepared and inserted into the subcutaneous tissue pockets of the nasal dorsum of each rabbit. The animals were sacrificed three and six months later, and graft specimens were examined histomorphologically using hematoxylin–eosin staining and Masson trichrome staining.

Results. Histomorphological analysis revealed various important findings of the cartilage and surrounding tissues according to the different types of costal cartilage. The thick cartilages exhibited decreased thickness compared with the thin cartilages over time (p = 0.038). Additionally, the thick cartilages showed a lower degree of vascularization than the thin cartilages at 3months (p < 0.001). A comparison of the cartilage forms revealed that the diced cartilages had better chondrocyte survival than the solid block cartilages (p < 0.001). Fat tissues were prominently observed surrounding the diced cartilages at 3 months (p = 0.01), and fibrosis was more prominently observed in the crushed cartilage than in the other types of cartilages (p = 0.04 and p = 0.005 at 3 and 6 months, respectively).

Conclusions. This study revealed differences in resorption depending on the thickness of the costal cartilage in rabbits for the first time. Among the various forms of costal cartilages, diced cartilage and thin solid block cartilage showed favorable outcomes of chondrocyte viability and growth compared with the other forms. These findings may help select the optimal type of costal cartilage for dorsal augmentation in rhinoplasty.

Keywords. Rhinoplasty; Costal cartilage; Diced cartilage; Resorption; Thickness; Viability

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Fig. 7 Surrounding fat tissues. (A) 3 months, 2-mm cartilage block, (B) 3 months, 1-mm

INTRODUCTION

Rhinoplasty is one of the most frequently performed cosmetic surgeries,¹ and autogenous cartilage grafts are commonly used in rhinoplasty. Various types of cartilages, such as septal, conchal, and costal, have been employed. Among them, costal cartilage is a preferred source of graft material owing to its robust strength and ample volume when a substantial amount of dorsal augmentation is needed.^{2,3}

Autologous costal cartilage can be used as a dorsal implant in various forms, e.g., solid block, diced, laminated, and crushed. However, each technique involves advantages and drawbacks.⁴ The use of solid block cartilages for dorsal augmentation is ideal, but some problems exist, such as warping, graft movability, resorption, infection and visible contour irregularity.⁵ To overcome the disadvantages of using solid block cartilage, the use of diced cartilage for dorsal augmentation as an alternative is gaining popularity. The main advantages are that it can be easily prepared and fine adjustments can be made without necessitating the harvest of a long piece of cartilage. Furthermore, diced cartilage has low potential for resorption. However, it is difficult to deliver to the nasal dorsum, which serve as major disadvantages.⁶

Numerous experimental studies have focused on diced and crushed cartilages and their graft survival following various manipulations.⁷⁻¹³ To the best of our knowledge, no study has directly compared the graft survival of solid, diced, and crushed cartilages and changes in their surrounding tissue over time. Furthermore, no study has investigated the degree of cartilage resorption according to the thickness.

Therefore, the primary goal of this study was to investigate cartilage viability according to the thickness and manipulations, and the secondary goal was to determine how various forms of costal cartilage differentially affect the surrounding tissues over time.

MATERIALS AND METHODS

Ten New Zealand white rabbits weighing 2,000–3,000 g each were used in this experimental study. All procedures were conducted in accordance with the ethical principles and guidelines for experiments on animals approved by the Asan Medical Center Institutional Animal Cares and Use Committee (2018-13-018). The surgical procedures were performed following the intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg) for anesthesia induction.

Surgical techniques

1. Cartilage harvest. The nose and anterior chest wall of the rabbits were shaved. The skin was prepared with povidone–iodine solution and draped in a sterile manner. A 2.5-cm vertical incision was made above the xiphoid area, and the seventh rib cartilage (thickness, ~2 mm; length, 2 cm) was harvested with its perichondrium due to it's greatest thickness, rabbit's costal cartilage from the true ribs has an average thickness of 2.18 ± 0.018 mm.¹⁴ (Fig. 1). The incision was closed using 4/0 vicryl sutures.

2. Graft preparation. The excised cartilage with its perichondrium was divided into four equal pieces (0.5 cm each) and manipulated into four different types: 2-mm block, 1-mm block, diced (0.1–0.15 mm in size), and crushed (moderately crushed¹⁵, two moderate-force hits to reduce the elastic strength enough to cause minimal bending downward with gravity and twisting by a delicate touch) Crushing was performed with a Cottle cartilage crusher (Karl Storz GmbH & Co, Tutlingen, Germany) (Fig. 2A).

3. Implantation of the cartilage grafts. A long vertical incision of approximately 5 cm in length was made at the top of the nose's skin of the rabbits, and the manipulated

cartilages were implanted 0.5cm apart under the skin as follows: 2-mm block, 1-mm block, diced, and crushed (Fig. 2B). The incisions were closed using 4/0 vicryl sutures.

4. Animal care. The respiratory function of the rabbits was monitored until they were ambulatory; they were then returned to their respective cages. A single shot of enrofloxacin, 10-mg/Kg body weight, was applied intravenously. All animals were provided with standard rabbit chow and water ad libitum.

5. Harvesting. The implantation sites of the rabbits were shaved again, and the cartilages were harvested after 3 months in five and 6 months in five. The cartilage specimens were fixed in 10% formalin solution. The animals were then sacrificed with overdoses of anesthetic materials.

Histopathological examination

The specimens were individually fixed in 10% formalin solution for 24 h, dehydrated in ethyl alcohol solution, and cleared using xylol. They were then embedded in paraffin blocks, cut into 5-µm-thick sections, and stained with hematoxylin-eosin (HE) stain and Masson trichrome stain. HE staining was performed to determine chondrocyte viability, chondroid tissue status, and changes in the surrounding tissues. Masson trichrome staining was performed to determine the collagen content of the matrix (collagen fibrils were stained blue) and fibrosis. The pathologist who performed the histological analysis was blinded to the examination groups. Direct cartilage thickness was measured using ImageJ software to evaluate the degree of cartilage absorption according to the thickness. The loss of chondrocyte nuclei was measured by direct calculation with a microscope at 400× magnification to evaluate the cartilage viability according to thickness. Four locations were randomly selected from the peripheral side of the cartilage, and the number of chondrocytes that lost their nuclei was counted via direct visualization. Vascularization was evaluated by counting the number of vessels around the cartilage. The amount of surrounding fat tissue (the ratio of the area occupied by fat tissue per total area measured) and degree of fibrosis (the average length of four areas at the symmetrically opposite side) were measured using ImageJ. Other parameters, including peripheral proliferation, matrix collagen contents, and inflammation, were difficult to quantitatively evaluate. Therefore, they were semiquantitatively recorded as percentages: 0% as none (-), 1%–25% as minimal (1+), 26%–50% as moderate (2+), 51%–75% as moderate-to-severe (3+), and 76%–100% as severe (4+).

Statistical analyses

Statistical analyses were performed using IBM[®] SPSS[®] Statistics for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). A nonparametric test (Kruskal–Wallis) and the Mann–Whitney U test were performed for intergroup comparisons. P < 0.05 was considered to denote a statistically significant difference.

RESULTS

Histomorphological analysis revealed various important findings of the cartilage and surrounding tissues according to the thickness and manipulations. The main features are as follows: (1) Cartilage thickness. The cartilage thickness in the 2-mm cartilage block at 6 months was significantly decreased compared with that at 3 months. There was a clear change in cartilage thickness over time. On the other hand, no tendency of change over time was noted in the 1-mm cartilage block (Fig. 3). (2) Loss of chondrocyte nuclei. At the same time points, the loss of chondrocyte nuclei was observed more in the 2-mm cartilage block than in the 1-mm cartilage block. At the same thickness, there was a change in the loss of chondrocyte nuclei over time in the 2-mm cartilage block but no significant difference in the 1-mm cartilage block. (Fig. 4). The diced cartilage showed lower loss of chondrocyte nuclei than the solid block cartilage (Fig. 5). In case of crushed cartilage, some grafts harvested at 3 and 6 months showed complete disappearance of cartilage tissues. (3) Vascularization. Vascularization surrounding the implanted cartilage was prominently observed in the diced and crushed cartilages. In terms of cartilage thickness, the 1-mm cartilage block exhibited more vessels in the surrounding tissues than the 2-mm cartilage block at 3 months. However, no significant difference in the number of vessels surrounding the cartilage harvested at 6 months was noted according to thickness (Fig. 6). (4) Surrounding fat tissue. There was a significant difference in the fat ratio among the groups in the cartilage harvested at 3 months (Fig. 7). Diced cartilage showed more surrounding fat tissue than the other types of cartilages. There was no significant difference in the surrounding fat tissue among the groups in the cartilage harvested at 6 months. (5) Fibrosis. A significant difference in the degree of fibrosis was noted among the groups (Fig. 8). Fibrosis was distinctively observed in crushed cartilage compared with that in the other types of cartilage. (6) Other parameters, such as peripheral proliferation, matrix collagen contents, and inflammation, were evaluated as a percentage and are shown in Tables 1 and 2. These parameters were

more distinct in the diced and crushed cartilages than in the solid block cartilage.

DISCUSSION

This study was conducted to determine whether cartilage resorption occurs, whether there is a difference according to the thickness of the cartilage tissue and manipulations, and whether various forms of cartilage (solid block, diced, and crushed) differentially affect the surrounding tissues over time. A significant difference in resorption according to cartilage thickness was found; the thickness of thicker cartilage tissues significantly decreased over time, but the thickness of thinner cartilage was not significantly changed. Additionally, changes in the surrounding tissues, such as vascularization, inflammation, and connective tissues (fat and fibrotic tissues), were more distinct in the diced and crushed cartilages than in the solid block cartilage.

Differences in resorption depending on the thickness of cartilage tissue are thought to occur because of the vascularization around the implanted cartilage. Cartilage has an avascular structure because chondrocytes receive nourishment via diffusion from the surrounding environment without direct bloody supply.¹⁶ Therefore, vascularization of the surrounding tissues may affect the resorption process of implanted cartilage. In the present study, 1-mm-thick cartilage harvested at 3 months showed more vessels in the surrounding tissues than 2-mm-thick cartilage. This difference was not observed at 6 months.

Similar results were obtained in terms of chondrocyte viability. Thicker cartilage showed more chondrocyte nuclei loss than that of thinner cartilage at a similar time point after direct counting using a microscope. This implies that a lower degree of vascularization in thick cartilage tissues affects chondrocyte viability and decreases its thickness. A significant change in chondrocyte nuclei loss in the 2-mm cartilage block was observed over time, but the change was not significant in the 1mm cartilage block (Fig. 3). This result is considered to be attributed to the similar thickness of thin cartilage tissues over time compared with that of thick cartilage tissues. In clinical practice, postoperative infectious complications including cartilage necrosis sometimes occur when using a thick costal cartilage for dorsal augmentation. It might be explained that the thick cartilage block has a lower degree of vascularization than the thin cartilage block.

Solid block and diced cartilage tissues have shown different results in previous experimental studies and clinical trials. In some studies^{12,13}, intact cartilage tissues showed significant viability and even new cartilage formation through proliferation. However, they exhibited massive destruction of the chondroid matrix leading to extensive loss of cartilage viability when diced or crushed cartilage grafts were used. Other studies have shown that the esthetic outcome of diced cartilage, especially when wrapped in fascia, is excellent and does not involve absorption. The studies concluded that the fascia has a cell-protective property.^{9,17-19} In our study, thin solid block and diced cartilages are both showed viable chondroid tissues and a lower degree of chondrocyte nuclei loss over time. It appears logical that diced cartilage grafts could maintain their viability. When the cartilage graft is diced, it will contain a high number of viable chondrocytes as a result of improved nourishment of small cartilage pieces.

In case of crushed cartilage, crushing intensity is a major factor that affects cartilage viability. As crushing intensity increased, cartilage viability decreased and more cartilage tissue was transformed into connective tissue.⁸ In our study, we did not compare the cartilage resorption according to the crushing intensity, but we crushed each cartilage under the same condition (two moderate-force hits to reduce the elastic strength enough to cause minimal bending downward with gravity and twisting with a delicate touch) to compare the degrees of resorption with a solid

block and diced cartilage. Among them, crushed cartilage exhibited severe cartilage resorption, and some of the crushed cartilages exhibited severe cartilage resorption as a result of transformation into fibrotic tissue.

In terms of surrounding tissue changes, vascularization of the tissues surrounding the implanted cartilage was prominently observed in the diced cartilage in the present study. When cartilage grafts are diced, their diffusion rate increases through the increased surface because more chondrocytes come into contact with the surrounding tissue. Therefore, there might be some driving forces of vascularization in the surrounding tissues. Further studies are needed to investigate the mechanism underlying the increase in vascularization. Fat tissues were significantly observed surrounding the diced cartilage. However, it is not clear whether the surrounding fat tissue is a result of fat infiltration into the empty space of diced cartilages or transformation into connective tissue as fat. However, some relationship may exist between chondrocyte survival and the surrounding fat tissues. In the present study, diced cartilage exhibited better chondrocyte survival and a significant fat ratio than the other forms of cartilage at 3 months. According to a recently published study²⁰, nanofat possesses regenerative cartilage capacity and was clinically demonstrated to relieve pain and repair damaged cartilage in patients with osteoarthritis. Further studies are needed to investigate the effect of fat tissue on chondrocyte survival. Since volume maintenance is important in dorsal augmentation, an increase in fat tissue around the implanted cartilage may be considered a favorable phenomenon in terms of volume maintenance.

In this study, we found strong evidence that diced cartilage and thin solid block cartilage were the best option for dorsal augmentation when considering longterm graft survival. Surgeons should focus attention on resorption according to thickness when using solid block cartilage for dorsal augmentation. The long-term survival of the graft is significantly preserved in thin solid block cartilage compared with that in thick solid block cartilage. Furthermore, multiple thin cartilage grafts can serve as an alternative if a large amount of solid block cartilage is needed for dorsal augmentation. Crushed cartilage may be another option for dorsal augmentation but is not recommended in large amounts for massive changes because the degree of resorption is not expected and affects the final outcomes of rhinoplasty.²¹

Our study has some limitations. First, many previous studies dealing with cartilage resorption directly measured the weight of the implanted cartilage. However, some of the implanted cartilage tissues exhibited weight gain after harvesting. Thus, it may not be appropriate to use this method to predict resorption. In our study, we directly measured cartilage thickness using Image J to evaluate resorption. Second, we directly counted the number of chondrocytes that lost their nuclei to observe chondrocyte viability. However, there are other techniques to more accurately measure chondrocyte viability via live/dead staining with confocal microscopy, and the results can be analyzed by specific imaging programs.²² This method should be taken into consideration in future studies. Third, the semiguantitative nature of histological parameters hindered statistical analysis. Therefore, peripheral proliferation, matrix collagen content, and inflammation were semiquantitatively evaluated by grade as a percentage. Forth, in clinical practice, a cartilage is usually used for grafting materials after eliminating the perichondrium. However, we implanted cartilage with its perichondrium in this study and compared the resorption between thick and thin cartilage blocks. Therefore there might be some doubt as to whether the results of this study could apply in clinical practice. However, it is clinically meaningful because we compared cartilage resorption under the same conditions.

CONCLUSION

In the present study, differences in resorption depending on the thickness of costal cartilage tissues in rabbits were noted. The thickness of thick cartilage significantly decreased with time, but that of thin cartilage was not affected by time and was relatively stable. Among the various forms of costal cartilages, diced cartilage and thin solid block cartilage showed favorable outcomes of chondrocyte viability and growth compared with the other forms. Changes such as inflammation, fat infiltration, fibrosis, and vascularization were more prominently observed in the tissues surrounding diced and crushed cartilage. These findings may help select an optimal technique of using costal cartilage for dorsal augmentation in rhinoplasty.

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Histological Parameters	2mm						1mm						Diceo	1		Crushed				
	n=5						n=5						n=5			n=5*				
	-	+1	+2	+3	+4	-	+1	+2	+3	+4	-	+1	+2	+3	+4	-	+1	+2	+3	+4
Peripheral proliferation	2	2	1	0	0	1	2	2	0	0	0	0	1	3	1	0	0	1	2	0
Matrix collagen	2	2	1	0	0	0	4	1	0	0	0	0	1	1	3	0	0	1	2	0
Inflammation	5	0	0	0	0	5	0	0	0	0	0	2	3	0	0	0	3	0	0	0

TABLE I.Histological Parameters of Groups (3months).

* Two grafts showed no cartilage tissues

 TABLE 2.

 Histological Parameters of Groups (6months).

Histological Parameters	2mm						1mm						Diced	1		Crushed				
i ulullotois	n=5						n=5						n=5			n=5*				
	-	+1	+2	+3	+4	-	+1	+2	+3	+4	-	+1	+2	+3	+4	-	+1	+2	+3	+4
Peripheral proliferation	3	0	2	0	0	0	4	1	0	0	0	0	2	3	0	0	1	1	2	0
Matrix collagen	0	1	2	0	0	1	3	0	1	0	0	0	2	3	0	0	0	1	3	0
Inflammation	4	1	0	0	0	4	1	0	0	0	0	4	1	0	0	1	1	2	0	0

* One graft showed no cartilage tissues



Fig. 1. Harvesting costal cartilage from a rabbit's anterior chest wall. The anterior wall of the rabbit was shaved (A, B). A 2.5-cm vertical incision was made above the xiphoid area (C). The seventh rib cartilage (thickness, \sim 2 mm; length, 2 cm) was harvested with its perichondrium (D, E, F).



Fig 2. (A) One set of four grafts (from right to left): 2-mm cartilage block, 1-mm cartilage block, diced, and crushed cartilage; (B) placement of the grafts.



Fig. 3. Cartilage thickness. (A) 3 months, 2-mm cartilage block, (B) 3 months, 1-mm cartilage block, (C) 6 months, 2-mm cartilage block, (D) 6 months, 1-mm cartilage block. A difference in resorption depending on the cartilage thickness was observed. The thickness of the thick cartilage significantly decreased with time (E), but that of thin cartilage did not show any difference (F).



E Loss of chondrocyte nucleus (3 months) F Loss of chondrocyte nucleus (6 months)











Fig. 4. Loss of chondrocyte nuclei. (A) 3 months, 2-mm cartilage block, (B) 3 months, 1-mm cartilage block, (C) 6 months, 2-mm cartilage block, (D) 6 months, 1-mm cartilage block. When compared at similar time points (E, F), the number of lost nuclei chondrocytes was significantly different between the 2- and 1-mm cartilage block. The difference was significantly observed only in the 2-mm cartilage block when compared at a similar thickness (G, H).



Fig. 5. Loss of chondrocyte nuclei according to the cartilage types. (A) At 3 months, (B) at 6 months. Diced cartilage showed a lower degree of chondrocyte nuclei loss than the solid block cartilage (crushed cartilage could not be evaluated because some grafts showed no cartilage tissues).



Fig. 6. Vascularization. (A) 3 months, 2-mm cartilage block, (B) 3 months, 1-mm cartilage block, (C) 3 months, diced cartilage, (D) 3 months, crushed cartilage. The surrounding vascularization of the implanted cartilage showed significant differences among the groups (E, F). In terms of cartilage thickness, the thin cartilage block showed more vessels than the thick cartilage block at 3 months (E) (*p < 0.001, statistically significant difference between the 2- and 1-mm cartilage block).





Fig. 7. Surrounding fat tissues. (A) 3 months, 2-mm cartilage block, (B) 3 months, 1-mm cartilage block, (C) 3 months, diced cartilage, (D) 3 months, crushed cartilage. Different fat ratios were observed among the groups at 3 months (E). However, this change was not significant at 6 months (F).



Fig. 8. Fibrosis. (A) 3 months, 2-mm cartilage block, (B) 3 months, 1-mm cartilage block, (C) 3 months, diced cartilage, (D) 3 months, crushed cartilage. Fibrosis of the surrounding tissue was mainly observed in crushed cartilage tissues (E, F).

배경 및 목적: 가슴연골의 경우 동양인의 코 성형에 있어서 콧등 이식물로 주로 사용되는 재료이며, 더 나은 결과를 얻기 위해서는 가슴연골을 어떠한 형태로 콧등에 이식할 것인가를 결정하는 것이 중요한 과제이다. 가슴연골을 사용하여 콧등에 이식할 경우 연골의 두께 및 가공 방법에 따라 흡수되는 정도가 다르며, 이는 코 성형의 최종 결과에 영향을 미치는 주요한 요인 중 하나이므로, 본 연구에서는 이식한 연골의 두께 및 가공 방법에 따른 연골의 흡수 정도 및 주변 조직의 조직학적 변화에 차이가 있는지 알아보고자 한다. 방법: 총 10 마리의 New Zealand White rabbits (aged 15-18 months and weighing 2400-3000 g)을 사용하여 2 cm 정도 길이의 가슴연골을 채취하여 5 mm 씩 4 등분으로 나눈 다음, 2 mm 두께의 단일 조각, 1mm 두께의 단일 조각, 다진 조각, 그리고 으깬 조각의 총 4 가지 형태로 토끼의 콧등 피부의 피하조직에 일정한 간격으로 심는다. 3, 6 개월 후 각각 5 마리의 토끼에서 심어놓은 연골 및 주변 조직을 채취하여 연골의 흡수 정도 및 조직학적 변화를 평가한다. 결과: 2 mm 두께의 단일조각의 경우 시간의 흐름에 따라 통계적으로 유의하게 두께의 감소가 관찰되었으며 (p = 0.038), 1 mm 두께의 단일조각의 경우 시간의 흐름에 따라 두께의 변화가 거의 관찰되지 않았다. 연골 주변의 혈관 분포의 경우 2 mm 두께의 단일조각에 비해 1 mm 두께의 단일 조각에서 더 많은 것으로 관찰되었으며, 이는 3 개월에 채취한

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연골에서만 통계적으로 유의한 차이가 관찰되었다 (p < 0.001). 3 개월과 6 개월의 동일 시점에서 비교했을 때 핵이 소실된 연골세포의 수는 2 mm 두께의 단일조각에서 1 mm 두께의 단일조각 보다 통계적으로 유의하게 더 많은 것으로 관찰되었으며 (p = 0.001), 동일한 두께에서 시간의 흐름에 따라 핵이 소실된 연골세포의 수는 2 mm 두께의 단일 조각에서만 통계적으로 유의하게 증가된 것으로 나타났다 (p = 0.002). 연골의 형태별로 시간의 흐름에 따라 비교하였을 때는, 다진 조각이 단일 조각 보다 핵이 소실된 연골세포의 수가 더 적은 것으로 관찰되었다 (p < 0.001). 연골 주변의 지방조직의 경우 다진 조각에서 가장 많은 비율로 관찰되었으며 (p = 0.01), 연골 주변의 섬유화 정도의 경우 으깬 조각에서 3개월 (p = 0.04)과 6개월 (p = 0.005) 모두 가장 높은 것으로 나타났다. 결론: 토끼의 가슴연골을 이용한 본 연구 결과, 가슴연골의 두께에 따라 연골의 흡수 정도의 차이가 있다는 것이 관찰되었고, 여러 가지 형태의 가슴 연골 중 두께가 얇은 단일조각과 다진 조각의 형태가 연골 세포의 생존에 있어서 유리한 결과를 보여주었으며, 이러한 연구 결과는 코 성형에 있어서 콧등 이식물의 재료로 가슴연골을 사용할 때 어떠한 형태로 사용하는 것이 가장 좋은 방법인지에 대한 판단에 도움을 줄 수 있다.

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