Costal cartilage graft with perichondrium, a possible anti-adhesive material

ORIGINAL ARTICLE

Norio Fukuda, Hirotaka Asato, Kohei Umekawa, Goro Takada, Takeshi Kan, Shoichi Sasaki

Department of Plastic and Reconstructive Surgery, Dokkyo Medical University, Tochigi, Japan

Correspondence: Norio Fukuda, MD, Department of Plastic and Reconstructive Surgery, Dokkyo Medical University, 880 Kitakobayashi, Mibu-Machi, Shimotsuga-Gun, Tochigi, 321-0293, Japan. Tel: +81 282 87 2485. Fax: +81 282 86 1806. E-mail: n-fukuda@dokkyomed.ac.jp

Abstract

Background: Adhesion occurs as a part of wound healing process, but it sometimes compromises patients' daily activities. We were looking for materials and methods that could prevent adhesion, and noticed that the costal cartilage has its possibility. We examined histologically the anti-adhesive property of the costal cartilage. *Methods:* Thirty-five patients with microtia who provided consent for participating in this study were enrolled between April 2008 and March 2015. In the first stage of microtia reconstruction surgery, we used the excess cartilage to create these three types of specimens: A) a piece of cartilage retaining the perichondrium on one side, B) piece of only cartilage parenchyma sliced with a plane parallel to the long axis of costal cartilage, C) the costal cartilage in a plane perpendicular to the long axis sliced pieces. These specimens were implanted into the subcutaneous fat of the chest. After at least 6 months in the second stage of surgery (i.e., auricular elevation), we took out these specimens wearing a little around the adipose tissue and examined them histologically. *Result:* A fibrosis formation of perichondrium side of specimen A was thicker significantly than that of cartilage side. A fibrosis formation of specimen B was thicker significantly than that of cartilage side of specimen A. *Conclusion:* It was suggested that if there is perichondrium, costal cartilage parenchyma surface makes less adhesion with surround

tissues. Costal cartilage with unilateral perichondrium is likely to be effective surgical materials for adhesion prevention.

Key Words: Costal cartilage, perichondrium, adhesion, anti-adhesive material, microtia

Introduction

In our institution, microtia reconstruction surgery is performed with two-stage surgical technique. In the first stage, costal cartilage segments are harvested to fabricate an ear framework and the remaining cartilage pieces are buried subcutaneously for the second stage surgery [1]. In the second stage, the cartilage pieces which are buried in the first stage are re-harvested and used as a buttress for framework elevation.

We observed that when the cartilage was re-harvested, the portion without perichondrium tended to form scarce adhesion with surrounding tissue. The encapsulated appearance is similar to that seen around implanted artificial materials (e.g., silicone device). On the contrary, the portion with perichondrium tended to form strong adhesion (Figure 1).

From these experiences, we assumed that the costal cartilage has anti-adhesive property and examined it histologically.

Materials and Methods

Thirty-five patients (20 men and 15 women) who underwent microtia reconstruction surgery from April 2008 to March 2015 were enrolled (Table 1). The mean patient age was 11.9 years (range, 9-27 years).

In the first stage of surgery, the sixth, seventh, and eighth costal cartilages were harvested to fabricate an ear framework. The cartilages used as a buttress for auricular elevation in the second stage of surgery were buried in subcutaneous fat of the chest. The extra cartilages, which were usually discarded, were used to create three types of specimens: Specimen A, a costal cartilage with unilateral perichondrium, sliced parallel to long axis of cartilage (5 mm × 8 mm × 2 mm in thickness) ; Specimen B, a piece of cartilage only, which was made by slicing the costal cartilage longitudinally without perichondrium (5 mm × 8 mm × 2 mm in thickness) ; Specimen C, a piece of cartilage, which was made by slicing the costal cartilage with removal of the perichondrium vertically against the longitudinal axis (2 mm in thickness) (Figure 2). These three pieces were marked with a thin, black nylon thread 10 mm in length for detecting and retrieving them easily and buried in the subcutaneous fat of the chest, separately from the cartilages that were to be used as a buttress in the second stage of auricular elevation

surgery (Figure 3).

At least 6 months after the first stage, the buried cartilages were retrieved for the second stage of surgery and at the same time, Specimens A, B, and C were retrieved with the surrounding adipose tissue (Figure 4). This adipose tissue is likely to peel off from the specimens, so we retrieved the specimens carefully. The specimens were fixed in 10% formalin and embedded in paraffin without decalcification. The paraffin-embedded specimens were cut at 3μm in thickness and stained with hematoxylin eosin and Masson's trichrome. Note that each specimen was prepared for section by slicing vertically against the center part of it. The light microscopic histology images were imported into a personal computer. We used Adobe Photoshop® (Adobe Systems Inc., San Jose, CA) for examination. We defined the layer that existed among the adipose tissue and perichondrium (Specimen A) or cartilage (Specimen B, C, and opposite side of A) as fibrotic layer. When we measure this area, we always confirmed the tissue with high power magnified images of the specimens dyed by hematoxylin eosin and Masson's trichrome, and assessed the margin of fibrotic layer. We examined the fibrotic area adjacent to the margin of the specimen along whole surface in the section and marked this layer manually. However, we were occasionally a little difficult to understand the boundary between perichondrium and fibrotic layer in Specimen A. In this situation we

found cells of perichondrium which are crowded densely side by side. We determined the boundary between perichondrium and fibrotic layer as the outer margin of cells in perichondrium (Figure 5). The area of fibrotic layer was measured on the captured image by the measurement tool of Photoshop®. We divided the area by the length of the contact surface border. Then, the fibrotic area per 1μm margin of the specimen was calculated. In the Specimen C, an axial section of costal cartilage we made three equal parts, in a central part and two surface parts and we calculated the fibrotic area per 1μm by each of central part and surface part for comparing the fibrosis between the both parts.

Statistical analysis

Calculated data were compiled using Microsoft Excel®. Data are presented in numbers (n), means (M) and standard deviations (SD).

Statistical analysis was performed using StatMate® (ATMS Inc., Tokyo, Japan) version 5 for Windows®. Student t-test was used for between-group comparisons. Values of $p < 0.05$ were considered statistically significant.

Ethical considerations

Note that the examined cartilage pieces were those which are usually discarded in

microtia surgery and were not harvested only for the present study. The preserving method of specimens in the chest wall is also similar to that of routine microtia surgery. This study was approved by the Ethics Committee of Dokkyo Medical University Hospital (the review number: 23007). All patients and their parents received verbal and written information about the study before giving their written consent to participate.

Results

The mean period of subcutaneous preservation of the specimens was 281.8 days in 35 patients.

The clinical and measurement data are shown in Table 1. In some samples, the adipose tissue was detached from the specimen and the cartilage surface was exposed during the sampling procedure. Such specimens were excluded from the study because an accurate assessment was not expected (represented by "–" in Table 1). Even if cartilage surface was not exposed directly, we occasionally noticed a slight stripping region under a microscopic examination. We thought that the dissection process will probably yield artifact and the data was excluded in such region.

For Specimen A, the fibrosis area of the side with and without the retention of the perichondrium was measured. The mean fibrosis area of the side with the

perichondrium was 352.8 (± 253.0 SD) μm^2 per 1μm perichondrium. The mean fibrosis area of the side without the perichondrium was 116.0 (± 157.5 SD) μm^2 per 1μm cartilage. The fibrosis of the side with the perichondrium was significantly greater than that of the side without the perichondrium (the statistical difference in the means was determined by using the one-sided paired t test) (Figure 6).

The entire surface of Specimen B was the cartilage parenchyma. The mean fibrosis area was 269.0 (± 242.1 SD) μm^2 per 1μm cartilage. The mean fibrosis area in the Specimen B group was significantly greater than that of the side without the perichondrium in the Specimen A group (the statistical difference in the means was determined by one-sided unpaired t test).

Specimen C was used for comparing the fibrosis between the surface and the central part of the costal cartilage. The mean scarring area of the surface was 212.9 (± 188.9 SD) μm^2 per 1μm cartilage; the mean fibrosis area of the central part was 314.9 (± 320.9 SD) μm^2 per 1μm cartilage. There was no significant difference in the fibrosis areas between the surface and the central part of the costal cartilage (the statistical difference in means was determined by two-sided paired t test).

Discussion

Adhesion is formed during the wound healing process. Beneficial adhesions are created intentionally as preventive or corrective means in certain procedures (e.g., gastropexies, herniorrhaphies). However, on the other hand, adhesive scar formation compromises patient's daily activities in some situations such as tendon adhesion after tendon repair. Despite advances in medicine, the prevention of undesired adhesion is still challenging.

Recently, many kinds of artificial materials are used to prevent adhesion especially in tendon surgery [2-8]. However, an artificial object may result in the development of adverse effects including infections, exposures, and deviations. None of them are applied clinically as routine permanent use.

Since an autograft tissue rarely causes those problems, an autograft tissue with anti-adhesive property would be an ideal material for adhesion prevention. We hypothesized that the costal cartilage had the anti-adhesive property from our experience in microtia reconstruction surgery.

Previous reports on adhesion formation of costal cartilages

Most of the previous reports on costal cartilages and the perichondrium discussed the regeneration of cartilage and absorption of the implanted costal cartilages [9-13]. Irkören et al. conducted an experimental study with rabbits and reported that less adhesion

occurred when the auricular perichondrium graft was wrapped around the Achilles tendon compared to the control group [14]. To the best of our knowledge, there is no study focusing on the adhesion formation of the implanted costal cartilage in humans.

The result of the present study

In the specimens retaining the perichondrium on one side (i.e., Specimen A), the side with the perichondrium showed more severe fibrosis than the contralateral cartilage parenchyma side (Figure 7). This could be observed because the nutrition and oxygen are mainly supplied to cartilage through perichondrium [15-17].

A little fibrosis formation was expected on the entire surface of the specimens without perichondrium (i.e., Specimen B). However, as a result, the cartilage was encapsulated with fibrous tissue and showed strong fibrosis with adjacent tissue. In addition, the angiogenesis was observed in some portions of these specimens. Contrary to the expectation, fibrosis was observed (Figure 8).

Interestingly, the cartilaginous parenchyma side of the cartilage with the contralateral perichondrium showed less fibrosis formation than the cartilaginous parenchyma side of the cartilage without any perichondrium. We assumed that a part of cartilage creates adhesion with surrounding tissue to get nutrition and oxygen when the

cartilage does not retain any perichondrium.

About chondrocytes, it is said that their morphology and function are different by the depth that exist. We expected that a difference also might be reflected on fibrosis by depth. Therefore, we made Specimen C, which was sliced against the longitudinal axis of costal cartilage for comparing the scarring fibrosis between the surface and the central part of the costal cartilage. However, there was no significant difference in the scarring fibrosis areas between the surface and the central part of the costal cartilage. In this study, the effect of the polarity of cartilage on fibrosis formation was not observed.

Note that in this study we used the parameter just measurements along one linear dimension, but our specimens are really three-dimensional structures, so we think continuous sections and mathematical integration are required originally for accurate analysis. However, it is difficult practically. When we previously cut several specimens and examined at multiple sections, we knew that there is not a big difference by a part. So we decided to adopt data by a section at a central part of specimens as representative value of each specimen.

The future prospects and applicability

A costal cartilage covered only one side with perichondrium may have the anti-adhesive

property on the contralateral side to perichondrium. If this property is clinically applicable, a costal cartilage with unilateral perichondrium could be used as a graft for adhesion prevention. While the cartilaginous parenchyma side of costal cartilage has anti-adhesive property, the perichondrium side creates strong fibrosis with surrounding tissue, which reinforces the structural stability of implanted graft.

In hand surgery, especially for tendon sheath reconstruction where adhesion is unfavorable, various sources of graft material such as autogenous tendons [18, 19], extensor retinaculum [20, 21], and volar plate [22] are reported. The costal cartilage with unilateral perichondrium may be used in this situation. Moreover, for the reconstruction of a ductal architecture such as the lacrimal duct and salivary duct, this autograft has the advantages of cartilage support as a buttress in addition to the anti-adhesive property which may prevent luminal and salivary occlusion.

At present, the possible applications are limited because of the size of cartilage and perichondrium those can be harvested from human body. This limitation will be overcome with the advances in culturing technique of the cartilage and perichondrium.

Conclusion

This study suggests that strong fibrosis occurs on the cartilage with perichondrium and

scarce fibrosis develops on the cartilaginous parenchyma side of the cartilage retaining contralateral perichondrium. The costal cartilage graft with unilateral perichondrium can be used as an anti-adhesive material and further studies are needed to assess its clinical benefits.

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Declaration of interest: The authors declare that there is no conflict of interest relevant to this article. The authors alone are responsible for the content and writing of the paper.

Figures

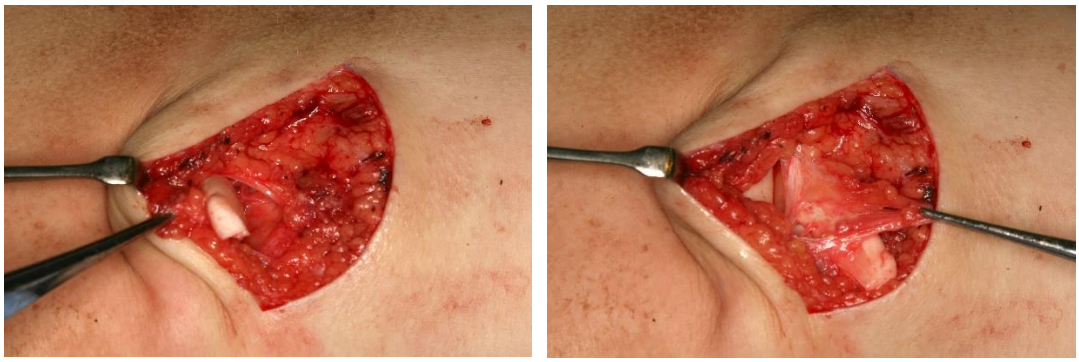

Figure 1. The buried cartilage pieces are retrieved. (a) The part without the perichondrium is found to scarcely adhere to the surrounding tissue. (b) The part retaining the perichondrium is found to densely adhere to the surrounding tissue.

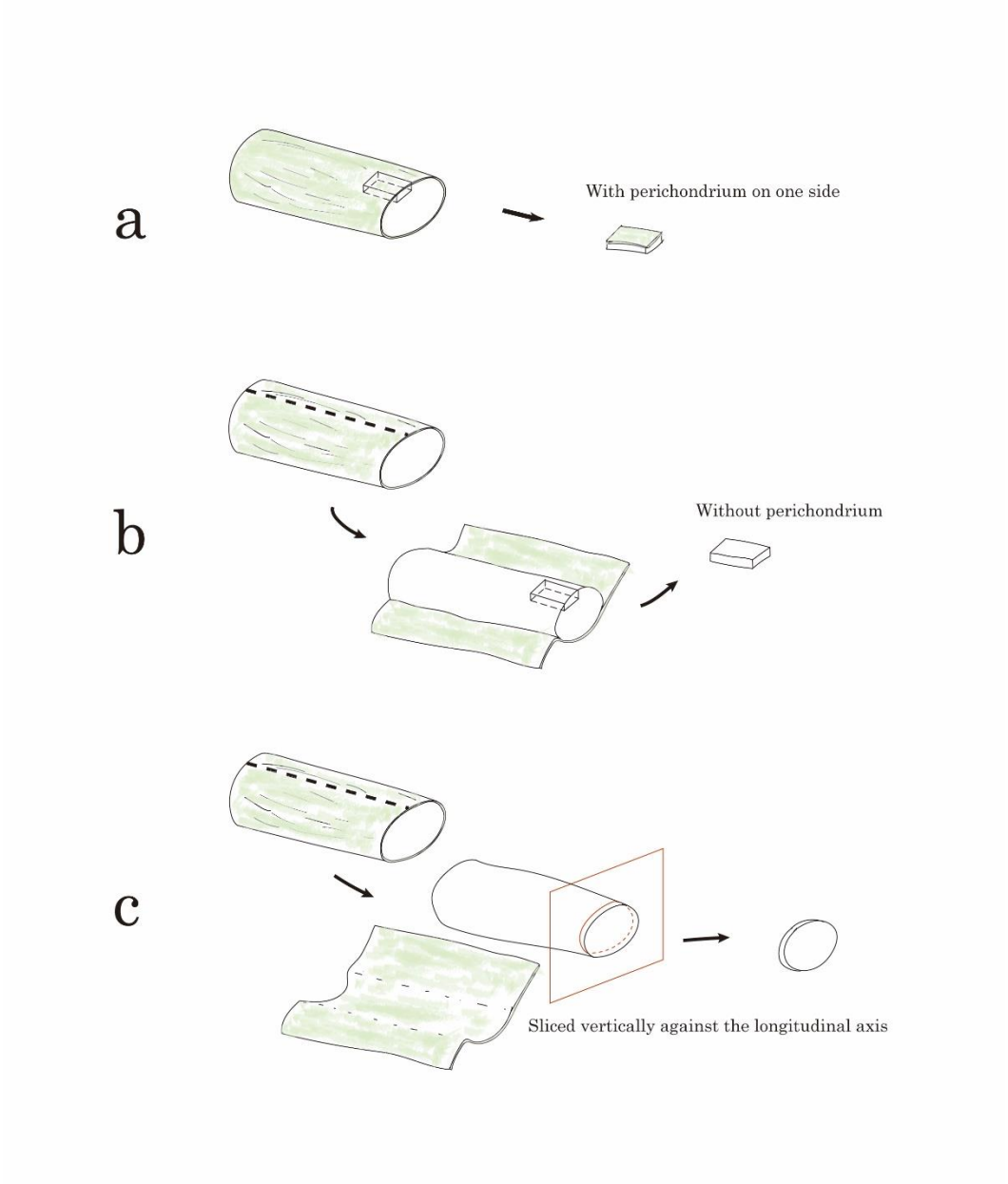

Figure 2. The specimens. (a) Specimen A: a piece of cartilage retaining the perichondrium on one side. (b) Specimen B: a piece of cartilage parenchyma without perichondrium. (c) Specimen C: a piece of cartilage, which was made by slicing the costal cartilage with removal of the perichondrium vertically against the longitudinal axis.

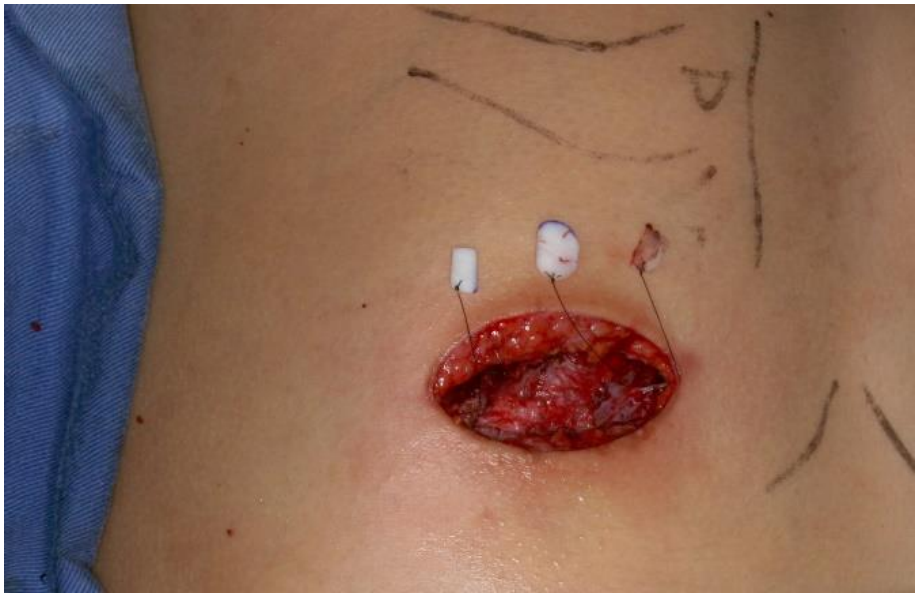

Figure 3. The specimens were preserved in the subcutaneous fat of the chest.

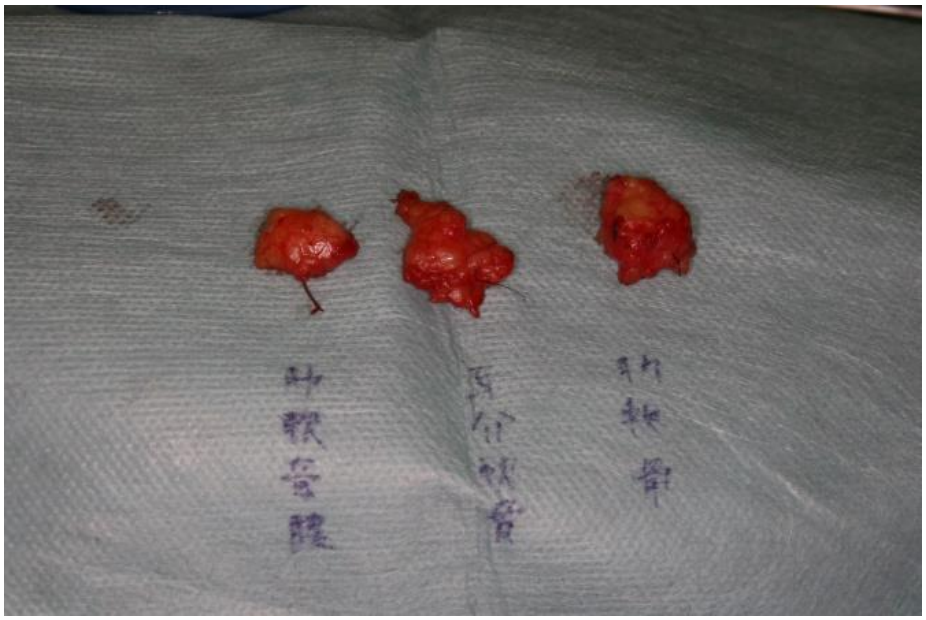

Figure 4. In the second stage of surgery (auricular elevation) we took out the specimens wearing a little around the adipose tissue.

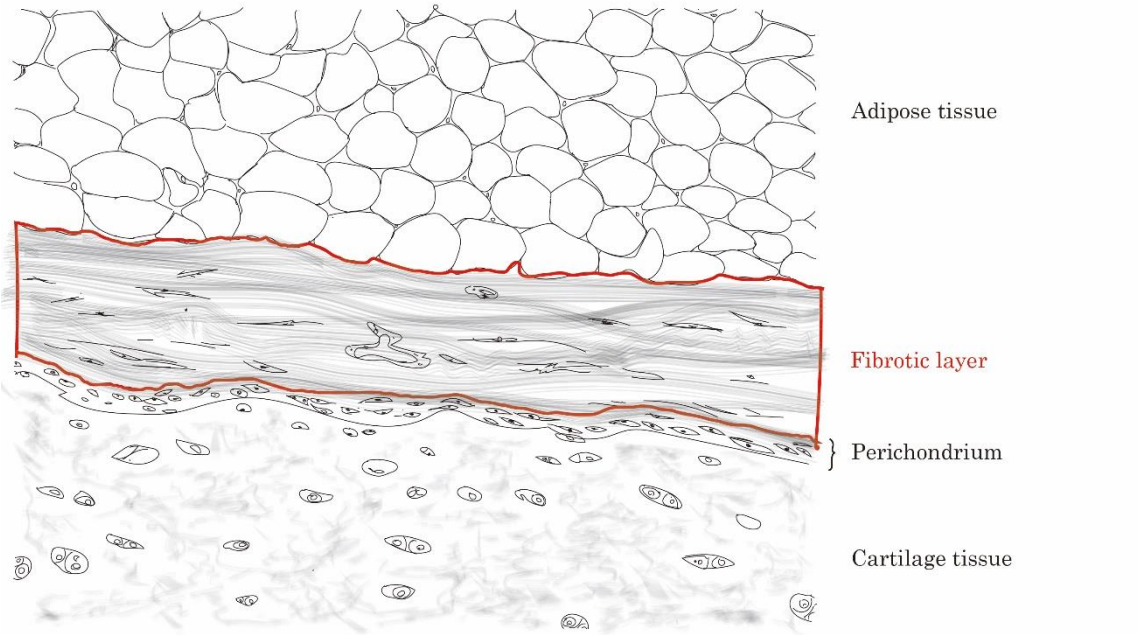

Figure 5. Fibrotic layer was defined that existed among the adipose tissue and perichondrium (Specimen A) or cartilage (Specimen B, C, and cartilage side of A).

We also determined the boundary between perichondrium and fibrotic layer as the outer margin of cells of perichondrium (Specimen A).

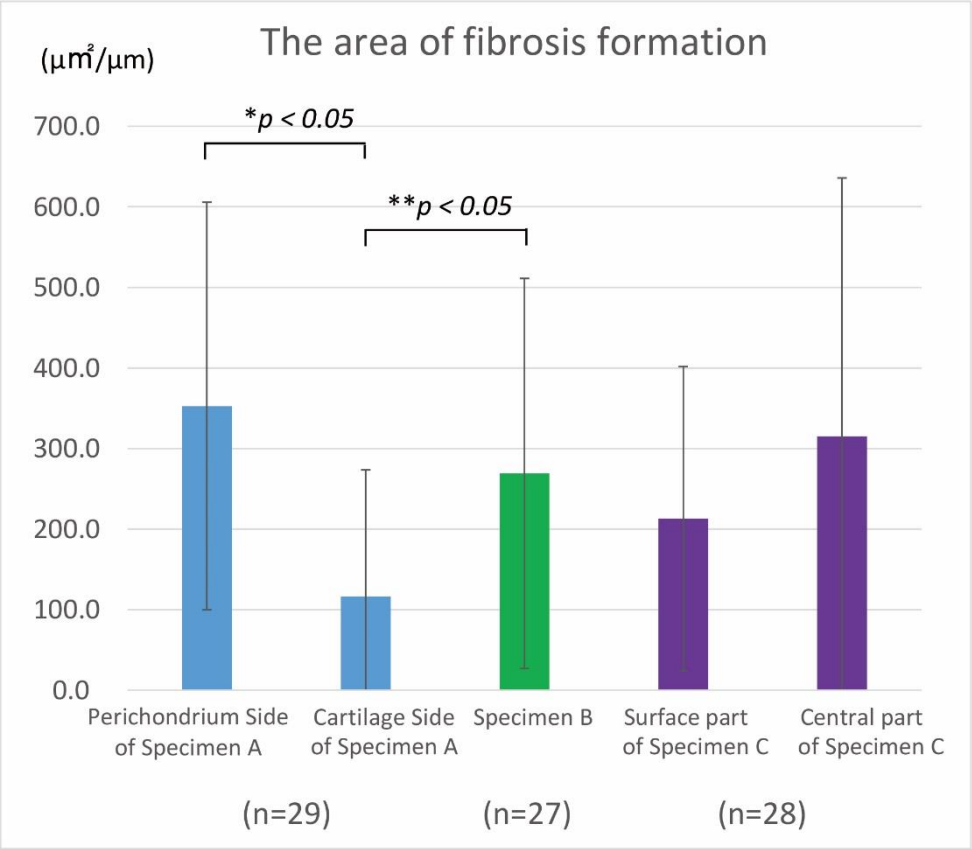

Figure 6. The area of fibrosis formation.

*used the dependent Student t-test.

**used the independent Student t-test.

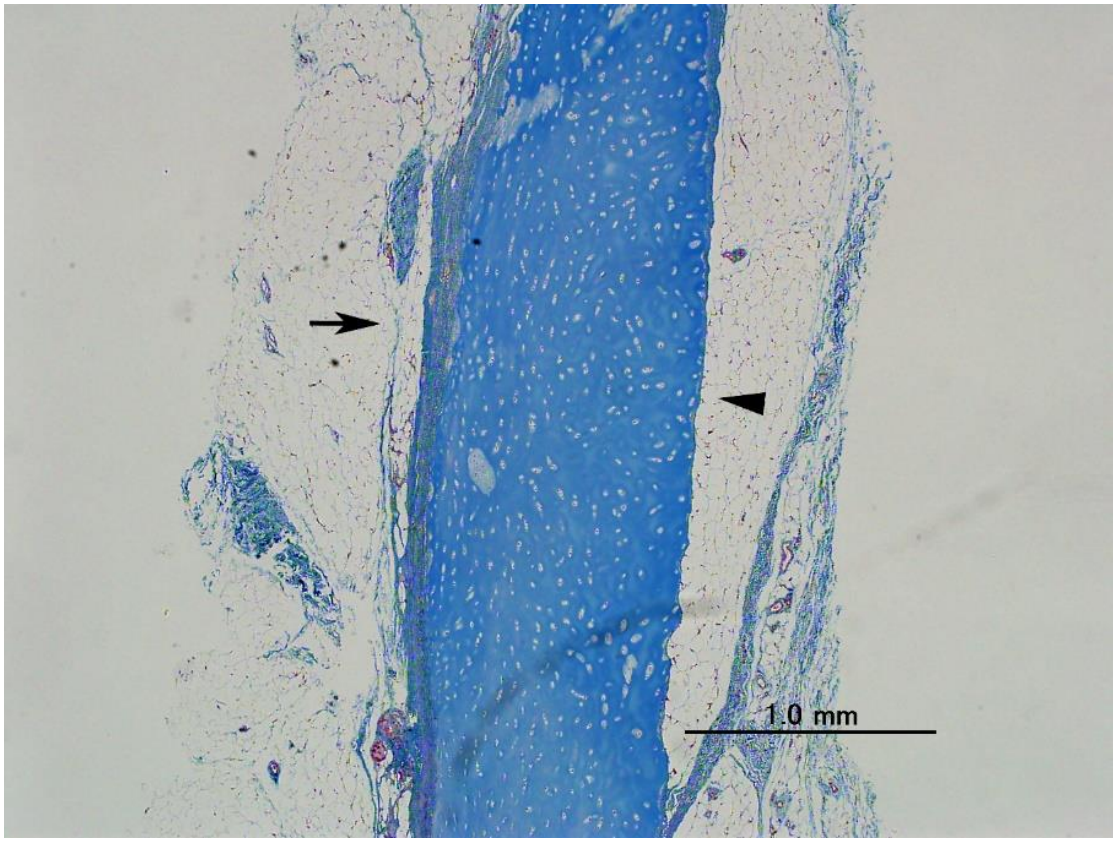

Figure 7. In the specimen A, fibrosis formation was observed strongly at the side of perichondrium, in contrast there were not any fibrosis at the side of cartilage. Arrow indicates perichondrium side, arrowhead indicates cartilage side (Masson's trichrome stain, ×4).

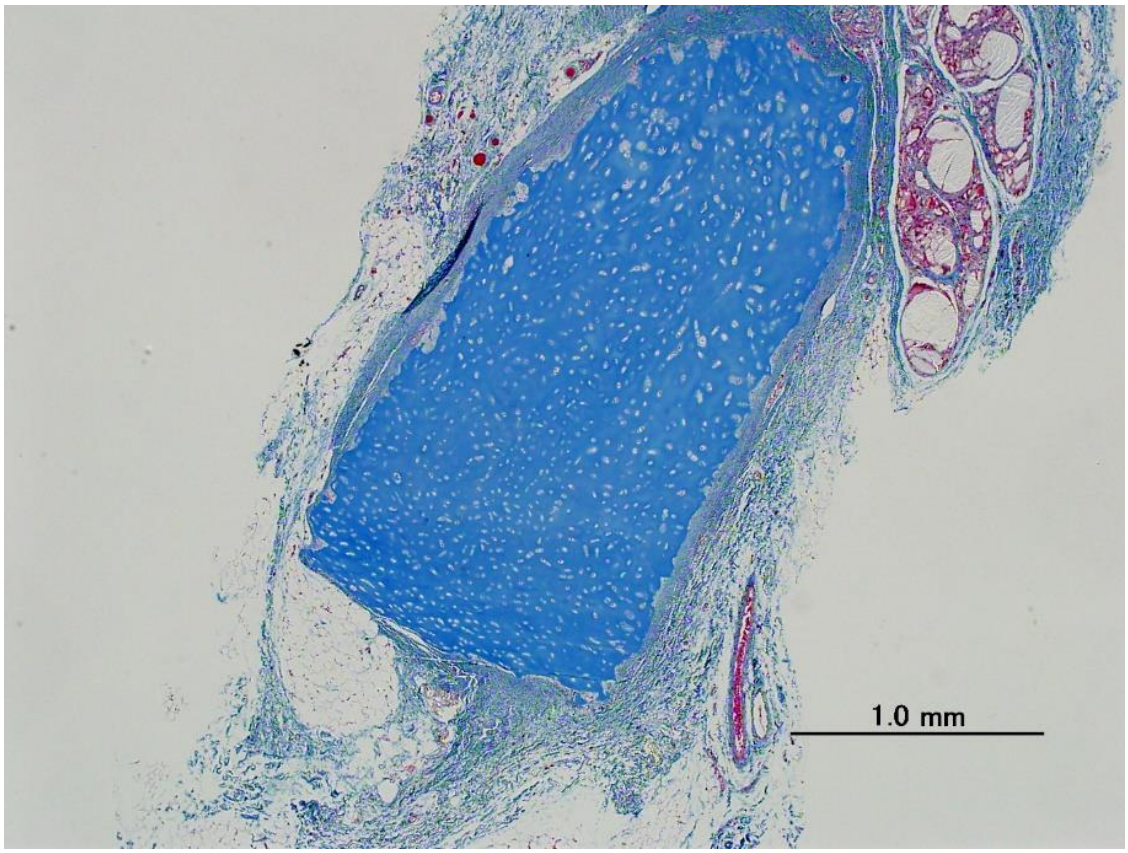

Figure 8. In the specimen B, thick fibrosis formation was observed at some parts of surface of cartilage (Masson's trichrome stain, ×4).

Table 1. Clinical data and results of the area of fibrosis formation with each surface of specimens.

Case	Sex	Age	Period of preservation (day)	The area of fibrosis formation with each surface of specimens calculated per 1um. (um^2/um)					
				Specimen A		Specimen B	Specimen C		
				Perichondrium side	Cartilage side		Surface part of the cartilage	Central part of the cartilage	
1	M	9	182	304	126	-	-	-	
2	F	11	469	118	131	21	-	-	
3	F	10	238	131	30	446	-	-	
4	M	16	294	-	-	118	-	-	
5	M	21	266	-	-	881	684	676	
6	M	10	245	258	37	-	760	286	
7	F	27	199	583	81	229	-	-	
8	M	10	195	469	29	-	18	633	
9	M	9	175	1198	890	489	227	72	
10	M	10	196	190	61	311	205	239	
11	M	10	357	355	173	512	-	-	
12	M	10	196	387	85	-	141	137	
13	F	16	532	434	168	76	101	108	
14	M	10	266	-	-	-	20	29	
15	M	16	175	433	42	65	267	710	
16	M	12	168	446	120	272	-	-	
17	M	11	167	-	-	256	224	72	
18	F	9	203	309	60	185	195	55	
19	F	10	273	200	81	105	33	35	
20	M	10	182	-	-	121	96	85	
21	F	11	385	548	56	85	53	1270	
22	M	10	252	284	201	157	99	330	
23	F	10	581	112	44	101	93	910	
24	M	11	308	850	68	289	535	348	
25	M	15	182	-	-	211	307	66	
26	M	10	280	75	26	295	369	428	
27	F	10	259	403	75	249	340	91	
28	M	10	238	85	59	-	284	52	
29	F	10	231	82	97	53	61	40	
30	F	10	224	234	48	-	234	381	
31	F	9	342	24	19	65	42	47	
32	F	13	217	617	33	83	42	234	
33	F	19	237	354	95	1010	209	719	
34	F	10	185	257	163	-	40	48	
35	M	10	945	390	266	578	271	717	
Means (M)		11.9	281.8	352.8	116.0	2680	212.9	314.9	
Standard deviations (SD)		3.9	152.1	253.0	157.5	242.1	188.9	320.9	

The data represented by “- “ was excluded from the study because the adipose tissue was detached from the specimen and the cartilage surface was exposed during the sampling procedure.

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