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Experimental study on maintaining the curvature of transplanted cartilage: Influence of the number of cartilage struts

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Summary *Background:* When auriculoplasty is performed for microtia, wire often becomes exposed during the long postoperative period. We have investigated other materials for fixing cartilage. We previously reported that absorbable sutures are more appropriate than wire. The present animal experiments investigated the reasons why fixation of transplanted cartilage is maintained when using absorbable sutures.
Methods: The costal cartilages of Sprague-Dawley rats were harvested, and three cartilage transplant models were prepared. After bending a costal cartilage into a U-shape, it was fixed by using only absorbable sutures as the control or was fixed by suturing one or two cross struts of cartilage to the U-shaped graft. Then the cartilages were subcutaneously transplanted into the backs of the rats. They were removed 8 weeks later, and the return rate of the bent cartilages was assessed.
Results: The return rate was 74.0%, in the suture-only group (control), 27.9% in the one-strut group, and 8.3% in the two-strut group. When the sites of contact between the U-shaped graft and the cartilage struts were observed by light microscopy, adhesion of the two cartilages by fibrous connective tissue was observed.

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Conclusion: U-shaped cartilage grafts demonstrated a smaller return rate when there was a larger contact area with the cartilage struts. Each strut was fixed by fibrous connective tissue at the contact site, thereby maintaining the shape of the graft. Thus, when creating a cartilage framework, it is important to fix the bent cartilage to the cartilage struts with a sufficiently large contact area.

Introduction

When auriculoplasty is performed for microtia, wires are usually used to fix the costal cartilage frames. The elasticity of cartilage causes it to return to the original shape[1] after being bent, and wire is believed to be a better material for fixation of curved cartilage grafts, as it is less likely to loosen.

In previous studies with a follow-up period ranging from 3 months to 9.5 years and follow-up for 2 years, respectively, extrusion of wires and sutures occurred in 1.63% and 4.85% of the patients.[2,3] Based on long-term data accumulated at our hospital since 1984, 19 out of 84 patients (22.6%) undergoing auriculoplasty with transplantation of costal cartilage showed postoperative exposure of steel wires.[4,5] When this happens, infection and inflammation may occur, causing resorption or deformation of the cartilage framework. According to Brent, 40 out of 600 patients (7%) followed up for 17 years had trauma to the reconstructed ear,[6] and we encountered a patient with burns to the reconstructed auricle following elevation of the temperature of the fixing wires in a sauna.[7] Furthermore, Rudderman et al. reported 5% reduction of cartilage volume by 90 days after surgery.[8] Therefore, we believe that it is not appropriate to use steel wires to fix cartilage grafts for reconstruction of the auricle because there is a high risk of subsequent wire extrusion due to loss of cartilage volume or trauma.[4,5]

Accordingly, we have investigated the use of various suture materials other than wires for fixing the costal cartilage framework, and we have assessed the changes after transplantation to determine the most appropriate material. When nylon thread was used, extrusion occurred in 2 out of 5 patients (40%).[5] In contrast, deformation of the auricle or exposure of the fixing material was not observed after use of absorbable sutures.[5] Therefore, fixation of the cartilage framework with absorbable sutures seems to be a safe and effective method of auriculoplasty.[4,5]

However, it remains unclear why bent cartilage does not return to its original shape when braided (multifilament) absorbable sutures are used for fixation. All absorbable sutures lose at least 50% of their strength within 4 weeks of implantation.[9] In a comparative study of various absorbable sutures, the tensile strength of 4-0 polyglycolic acid (PGA) absorbable sutures was approximately 3.1% after 14 days.[10] The manufacturer's information for the Opepolix® PGA absorbable sutures (Alfresa Pharma Industries, Ltd., Osaka, Japan) used in this experiment states that the residual in vivo tensile strength after 28 days should be 3.4%; these sutures are completely absorbed, and they disintegrate by hydrolysis after a minimum of 8 weeks.

Because we consider that absorbable thread is the most appropriate fixation material for cartilage, we performed the present study in rats using PGA absorbable sutures (Opepolix®) to fix U-shaped cartilage grafts and investigated a method for preventing the grafts from returning to the original shape.

Materials and methods

Animals

All animal experiments in the present study were performed in accordance with Kurume University's Animal Experimental Regulations (approval number: 2015-098). Male Sprague-Dawley (SD) rats aged 12 to 14 weeks old and weighing 370-410 g were used.

Preparation of cartilage grafts

Following induction of anesthesia with diethyl ether (Wako Pure Chemical Industries, Ltd., Osaka, Japan), approximately 0.01 mL/kg per body weight of pentobarbital sodium (Somnopentyl®; Kyoritsu Seiyaku Corporation, Tokyo, Japan) was injected intraperitoneally. The rats were once again placed in a dark room and slept deeply for approximately 10 min, after which the hair on the chest and back was shaved with an electric razor, and each rat was immobilized on a laboratory table in the supine position.

Skin incisions were then made on the chest. After a central longitudinal incision was made over the sternum, oblique skin incisions were then made downward to the left and to the right along the lowermost margin of the thorax. The midline longitudinal incision was deepened into the muscle layer, so that the muscles could be lifted off the sternum and peeled back laterally over the costal cartilages. The skin was not removed from the muscle layer, and the flap was instead raised in one piece, considering blood flow. Harvesting of cartilage was started from the most-caudal costal cartilage of the thorax. Using tweezers, the cartilage was elevated slightly and the soft tissue along the cartilage was dissected. Careful dissection was required so that the pleural space was not opened. After dissection of 18-20 mm, scissors were used to detach the cartilage and it was placed in normal saline to prevent drying. If the pleural space was accidentally opened, the defect was promptly closed by sutures.

Three pieces of cartilage were harvested on each side in the cranial direction, for a total of six pieces (Figure 1). The three pieces of cartilage were harvested from the lower ribs because the upper ribs are more curved, and this increases

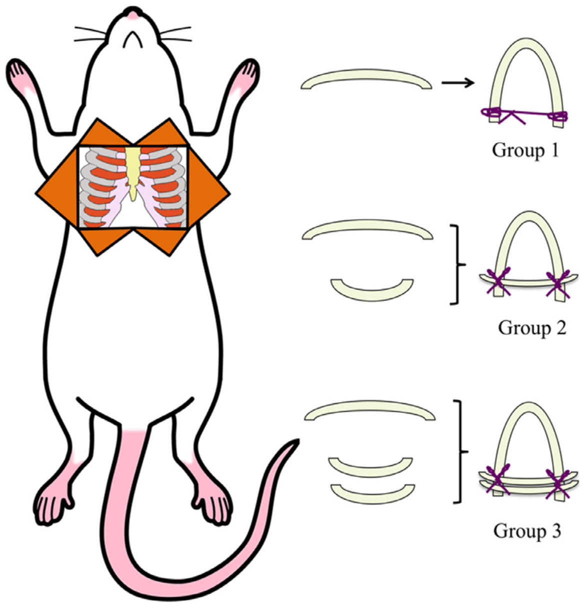


Figure 1 Harvesting cartilage pieces and creation of three transplant models. A total of six cartilage pieces were harvested from an SD rat. In Group 1, one piece of cartilage was bent into a U-shape and fixed with only absorbable sutures. In Group 2, one piece of cartilage was bent into a U-shape and fixed to a straight cartilage strut using absorbable sutures. In Group 3, one piece of cartilage was bent in the same way and fixed to two straight cartilage struts using absorbable sutures.

the risk of opening the pleural space with the risk of death. Muscle tissue and perichondrium attached to the harvested cartilage were removed as much as possible. After the cartilage was harvested, the muscle layers and skin were sutured to close the wound.

Among the six cartilages, three of the longer and less curved pieces were selected and were bent into a U-shape, while the other three pieces of cartilage were used as the cross struts (Figure 1). The distance between both ends of the piece of cartilage before bending was labeled A, and the distance between both ends of the bent and fixed cartilage was labeled B. The cartilage was bent and fixed so that the B/A ratio was approximately 1/2–1/3.

Three transplant models were tested. First, an assistant bent one cartilage into a U-shape toward the curved side, and both ends of the cartilage were fixed with absorbable sutures (4–0 Opepolix®). Then the distance between both ends was measured. This group with sutures only was designated as Group 1 and was the control group (Figure 1, upper right). Second, following the same procedure as that for Group 1, the cartilage was fixed using absorbable sutures. Then another piece of cartilage was placed as a cartilage strut between both ends of the bent cartilage, with the contact areas being fixed by absorbable sutures (4-0 Opepolix®). Criss-cross sutures were used so that the cartilage would not easily come loose. Subsequently, the suture used to fix both ends in the first step was cut, and the direct distance between both ends was measured. This cartilage group was labeled Group 2, the one-strut group (Figure 1, right middle). Third, after the same procedure as that in the one-strut group, one more cartilage strut was added to increase the contact area between the bent cartilage and the cartilage struts. This was also fixed with absorbable sutures, and the distance between both ends was measured. This cartilage group was labeled Group 3, the two-strut group (Figure 1, bottom right).

Cartilage transplantation

The prepared cartilage grafts were transplanted subcutaneously into the backs of the rats (Figure 2, left column). Because a rat may bite the sutures if the transplant site is close to the tail, a longitudinal incision was made on the back relatively close to the head (Figure 2, middle column). Then subcutaneous pockets were created on the left and right sides of the incision site by careful dissection to avoid hematoma formation. Cartilage grafts were placed into the pockets, the site of transplantation was recorded, and the skin was sutured to close the wound.

Removal of the transplanted cartilage grafts

The cartilage grafts were removed at 8 weeks after transplantation. The rats were anesthetized by the same procedure as that when the cartilage was transplanted, and the incision on the back was opened again. By palpation, we confirmed the location of the transplanted cartilages and extracted them with the surrounding soft tissues (Figure 2, right column). After extraction, the distance between both ends of each bent piece of cartilage was measured. The cartilage grafts that almost maintained the original shape were stored in centrifuge tubes with Bouin's solution and fixed for histological examination.

Calculation of the return rate and statistical evaluation

The distance between both ends of the cartilage before bending was labeled A, the distance between both ends of the bent and fixed cartilage was labeled B, and the distance between both ends of the removed cartilage at 8 weeks after transplantation was labeled C. The increase in the distance after 8 weeks (C-B) was calculated as percentage compared with the shortest distance just after bending (A-B) [C-B/A-B (%)], and this was defined as the return rate (Figure 3). The return rate for all removed transplanted cartilage grafts was calculated to evaluate statistical significance between the groups. Statistical evaluation was performed using JMP statistical software (SAS 11; SAS Institute Inc., Cary, NC, USA). One-way three-factor analysis of variance (ANOVA) was used, after which multiple comparisons were performed with Tukey's honestly significant difference test to compare the groups. Statistical significance was set at $P < 0.05$.

Processing of specimens for histological examination

The resected cartilage specimens were fixed in Bouin's solution for 6 h. After a brief rinse in phosphate-buffered

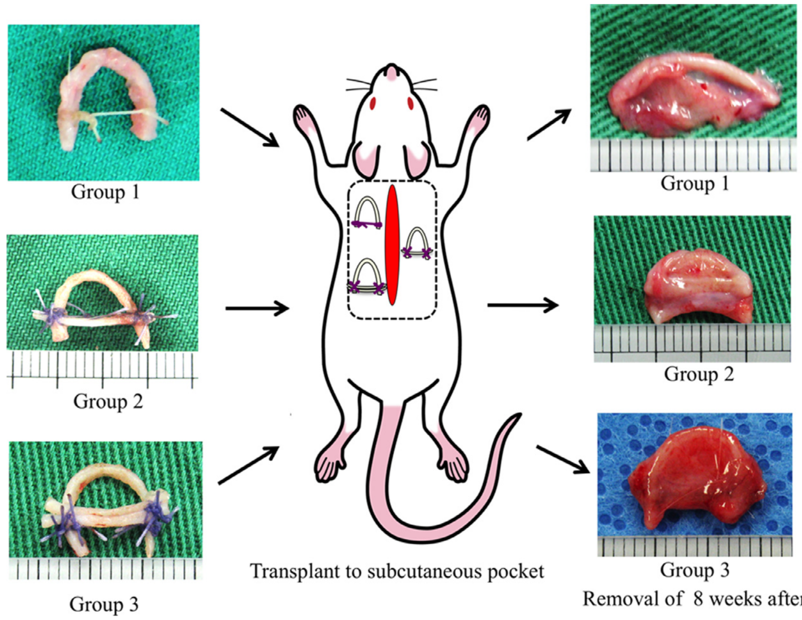
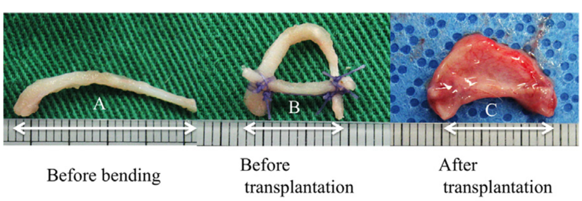


Figure 2 Transplantation of the three cartilage graft models and removal of transplanted grafts. Each cartilage graft model was placed into a subcutaneous pocket in the back of a rat. Then the implants along with the surrounding tissues were removed at 8 weeks after transplantation.



$$\text{Return rate (\%)} = \frac{C - B}{A - B}$$

Figure 3 Calculation of the return rate. The following distances were measured: direct distance between both ends of the cartilage before (A), direct distance between both ends of the bent and fixed cartilage (B), and direct distance between both ends of the cartilage removed at 8 weeks after transplantation (C). The return distance (C-B) was calculated as a percentage of the shortening in the distance between the cartilage ends achieved by bending (A-B) [C-B/A-B (%)], and this was defined as the return rate.

saline (PBS) (pH 7.4), the fixed tissues were immersed overnight in 30% sucrose solution (Nacalai Tesque, Inc., Kyoto, Japan) in PBS. Then the specimens were embedded in O.C.T. Compound (Tissue-Tec®, Sakura Finetek USA, Torrance, CA, USA). After freezing in liquid nitrogen, a microtome was used to cut tissue slices of approximately 5 μm in thickness. The sections were thoroughly dried on prepared slides, and toluidine blue staining and Masson's trichrome staining were performed. Then the contact area between the bent cartilage and the cartilage struts was observed by light microscopy.

Results

Comparison of the return rate

In Group 1 (the control group, $N = 9$), the distance between both ends before bending (A) was 17.0-20.0 mm (mean: 18.9 mm), the distance between both ends after bending (B) was 7.0-11.0 mm (mean: 8.0 mm), and the distance between both ends at 8 weeks after transplantation (C) was 15.0-19.0 mm (mean: 16.0 mm). Accordingly, the return rate was 41.7-88.9% (mean: 73.4%) (Table 1). In Group 2 ($N = 11$), A was 22.0-20.0 mm (mean: 20.9 mm), B was 8.0-11.5 mm (mean: 9.2 mm), and C was 10.0-14.0 mm (mean: 12.3 mm). As a result, the return rate was 0-41.7% (mean: 26.4%) (Table 2). In Group 3 ($N = 10$), A was 18.0-22.0 mm (mean: 20.1 mm), B was 4.0-9.0 mm (mean: 7.1 mm), and C was 5.0-10.0 mm (mean: 8.2 mm). Therefore, the return rate was 0-23.1% (mean: 8.4%) (Table 3). Statistical assessment revealed that the return rate was significantly smaller in Group 2 than in Group 1. Moreover, the return rate was significantly smaller in Group 3 than in Group 2 (Figure 4).

Histological examination

Histological examination of the transplanted cartilage grafts with toluidine blue staining revealed that the cartilage matrix was stained purple and the cartilage retained its properties after being bent. There was no fusion between the bent cartilage tissue and the cartilage struts, but fibrous connective tissue was formed alongside the components of the degraded absorbable sutures around the contact area of the two cartilage pieces (Figure 5). As this fibrous connective tissue was stained blue with Masson's trichrome stain, it

Table 1 Changes in cartilage length from pre-transplant to 8 weeks post-transplant in Group 1 (control group).

Section No.	Pre-bending; A (mm)	Pre-transplant; B (mm)	8 weeks post-transplant; C (mm)	Return rate (%)
1	17	8	16	88.9
2	18	7	15	72.7
3	20	8	18.5	87.5
4	17	5	10	41.7
5	20	11	19	88.9
6	20	10	18	88.0
7	19	8	15	63.6
8	19	5	16.5	82.1
9	20	10	16	60.0
10	-	-	-	-
11	-	-	-	-
Average	18.9	8.0	16.0	73.4

Table 2 Changes in cartilage length from pre-transplant to 8 weeks post-transplant, Group 2.

Section No.	Pre-bending; A (mm)	Pre-transplant; B (mm)	8 weeks post-transplant; C (mm)	Return rate (%)
1	22	8.5	12.5	29.6
2	20	11.5	14	29.4
3	20	10	10	0
4	20	10	14	40.0
5	22	8.5	11	18.5
6	22	9.5	14	36.0
7	22	9	13	30.8
8	21	10	14	36.4
9	21	8	10	15.4
10	20	8	13	41.7
11	20	8	10.5	12.5
Average	20.9	9.2	12.3	26.4

Table 3 Changes in cartilage length from pre-transplant to 8 weeks post-transplant, Group 3.

Section No.	Pre-bending; A (mm)	Pre-transplant; B (mm)	8 weeks post-transplant; C (mm)	Return rate (%)
1	20	7.5	8	4.0
2	20	6	6	0
3	20	7	10	23.1
4	20	8.5	10	13.0
5	22	8.5	9.5	7.4
6	22	8.5	10	11.1
7	20	9	10	9.1
8	18	6	7	8.3
9	19	6	7	7.7
10	20	6	6	0
11	-	-	-	-
Average	20.1	7.1	8.2	8.4

was clear that the cartilage pieces were fixed by an increase in collagen fibers, i.e., scar tissue (Figure 6).

Discussion

We investigated why absorbable sutures (Opepolix®), which maintain tensile strength only for 1 month,[11] were able to prevent postoperative deformation of the auricle after auriculoplasty. We originally hypothesized that this occurred because the bent cartilage tissue underwent structural changes or there were changes in chondrocytes within 2–3 weeks after transplantation. Accordingly, we expected that the shape of the bent cartilage would be maintained in Group 1 as well. However, at the time of extraction (8 weeks), most grafts in Group 1 had largely returned to the original shape (Figure 2, left column, upper row, right column, upper row). In other words, it seemed that when the strength of the absorbable sutures was lost at 1 month after transplantation, the structure of the cartilage had not

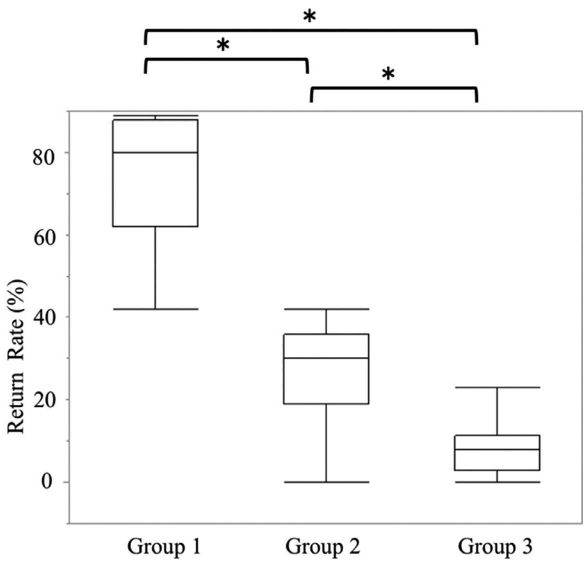


Figure 4 Comparison of return rates. Statistical assessment revealed that the return rate of Group 2 was significantly smaller than that of Group 1. Moreover, the return rate of Group 3 was significantly smaller than that of Group 2. One-way ANOVA Tukey's honestly significant difference test. $^{*}P < 0.05$.

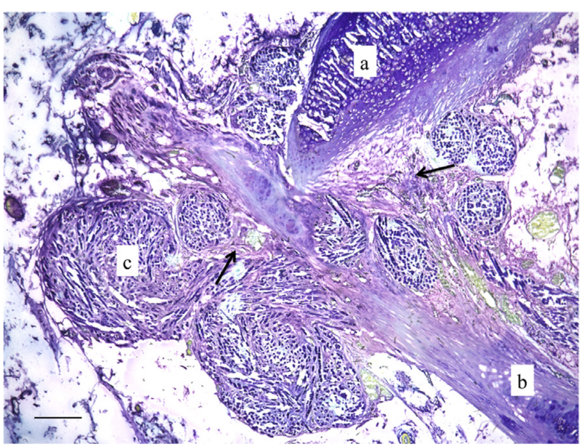


Figure 5 Toluidine blue staining of cartilage fixed with two struts after removal (8 weeks after transplantation). There were no fusion between the bent cartilage and the cartilage strut, but fibrous connective tissue was formed alongside the components of the degraded absorbable suture around the contact area of the two pieces of cartilage (arrow). (a): Bent cartilage at 8 weeks after transplantation. (b): Cartilage strut at 8 weeks after transplantation. (c): A degraded absorbable suture. Scale bar = 300 μm.

changed and it returned to its original shape because of elasticity. Therefore, our first hypothesis was rejected.

When making cartilage frameworks for microtia surgery, strongly bent cartilage such as that for forming the helix is securely fixed to cartilage struts. Therefore, our next hypothesis was that the bent cartilage maintained its shape due to adhesion to the cartilage strut by scar tissue. Therefore, we prepared cartilage transplant models with struts in the experiment. In brief, a straight piece of cartilage was fixed as a strut to both ends of a U-shaped bent cartilage. To provide variation in the contact area between the bent cartilage and the cartilage struts, we prepared models with one cartilage strut (Group 2) or two cartilage struts (Group 3). As a result, the return rate was significantly smaller in the two-strut group than in the one-strut group. In brief, as the contact area increased, the return rate of the bent cartilage decreased.

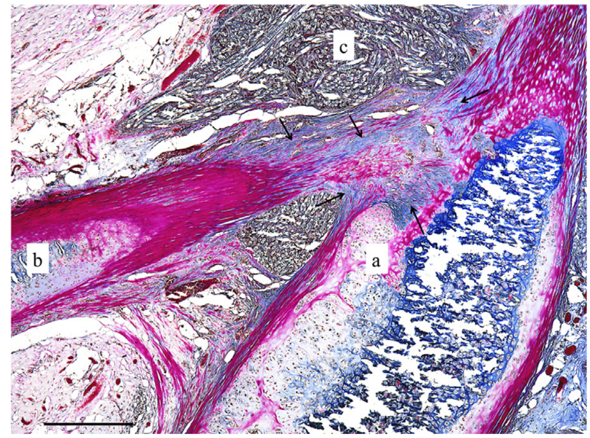


Figure 6 Masson's trichrome staining of the cartilage fixed with two struts after removal (8 weeks after transplantation). Collagen fibers between two cartilage pieces are stained blue. Both cartilages were fixed by the increase in collagen fibers. (a): Bent cartilage at 8 weeks after transplantation. (b): Cartilage strut at 8 weeks after transplantation. (c): A degraded absorbable suture. Scale bar = 500 μm.

Subsequently, to examine adhesion between the bent cartilage and the cartilage struts, we observed the contact area of the transplanted cartilage grafts in the two-strut group by light microscopy. Fusion of the cartilage tissue at the contact sites was not observed. This result was in agreement with the report that it is difficult for the cartilage to fuse with other cartilage.[12] Nevertheless, there was an increase in fibrous connective tissue around the contact area of the cartilage pieces, and the two cartilages were fixed by this tissue (Figure 6). Thus, the observation revealed that the cartilages were fixed by fibrous connective tissue, i.e., scarring at the contact area, and this was why the cartilage grafts maintained shape after the absorbable sutures lost tensile strength. It seemed that the bent cartilages were more securely fixed when there was a larger contact area between the pieces of cartilage. A limitation of the present study is that absorbable sutures were not compared with other fixing materials; hence, the contribution of absorbable sutures to fibrosis around the cartilage grafts could not be clarified.

However, our findings suggested that a large contact area with the cartilage struts is better when creating a cartilage frame by using absorbable sutures during microtia surgery, especially at sites where the cartilage has to be bent strongly, such as for forming the helix.

Conclusion

When considering the fixing material for creating cartilage frames during auriculoplasty, absorbable sutures are safer than wires or nylon thread, which frequently become exposed over the long term. As absorbable sutures lose strength over time after surgery, cartilage struts are required to prevent unfolding of bent cartilage. We emphasize that it is important to maintain a sufficiently wide contact area between the cartilage struts and the bent cartilage, especially when forming the helix or antihelix, because final fixation is achieved by the fibrous connective tissue (scar tissue).

Conflict of interest

None.

References

1. Gibson T, Davis WB. The distortion of autogenous cartilage grafts: its cause and prevention. *Br J Plast Surg* 1957;**10**:257.
2. Long X, Yu N, Huang J, Wang X. Complication rate of autologous cartilage microtia reconstruction: a systematic review. *Plast Reconstr Surg Glob Open* 2013;**1**(7):e57.
3. Zhou X, Zhang Q, Liu T, et al. Modification in the technique of ear framework fabrication for congenital microtia. *J Craniofac Surg* 2012;**23**(5):1296–300.
4. Tai Y, Tanaka S, Fukushima J, et al. Refinements in the elevation of reconstructed auricles in microtia. *Plast Reconstr Surg* 2006;**117**(7):2414–23.
5. Sakamoto A, Kiyokawa K, Rikimaru H, Watanabe K, Nishi Y. An investigation of the fixation materials for cartel frames in microtia. *J Plast Reconstruct Aesthet Surg* 2012;**65**(4):584–9.
6. Brent B. Auricular repair with autogenous rib cartilage grafts: two decades of experience with 600 cases. *Plast Reconstr Surg* 1992;**90**(3):355–74.
7. Rikimaru H, Tai Y. Therapeutic experience with reconstructed auricular burn by a wire. *Jpn J Plast Reconstr Surg* 2000;**20**:191–5.
8. Rudderman Rh, Guyuron B, Mendelsohn G. The fate of fresh and preserved, noncrushed and crushed autogenous cartilage in the rabbit model. *Ann Plant Surg* 1994;**32**:250–4.
9. Firestone DE, Lauder AJ. Chemistry and mechanics of commonly used sutures and needles. *J Hand Surg* 2010;**35**(3):486–8.
10. Khiste SV, Ranganath V, Nichani AS. Evaluation of tensile strength of surgical synthetic absorbable suture materials: an in vitro study. *J Periodontal Implant Sci* 2013;**43**:130–5.
11. Ireton JE, Unger JG, Rohrich RJ. The role of wound healing and its everyday application in plastic surgery: a practical perspective and systematic review. *Plast Reconstr Surg Glob Open* 2013;**1**:1–10.
12. Bonasia DE, Martin JA, Marmotti A, et al. The use of autologous adult, allogenic juvenile, and combined juvenile-adult cartilage fragments for the repair of chondral defects. *Knee Surg Sports Traumatol Arthrosc* 2016;**24**(12):3988–96.