Warping Characteristics of Rib Allograft Cartilage

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Summary: When septal cartilage is lacking, commercially available costal cartilage allograft can be used. Such allografts have "off-the-shelf" accessibility, are available in multiple sizes, are aseptically processed to meet sterility, and are screened to minimize infectious risks. The purpose of this study was to evaluate the effect of donor age, storage temperature, and orientation of a bilayered construct on the degree of warping of a commercialized fresh frozen costal cartilage allograft in vitro over time. A total of 140 fresh frozen costal cartilage cadaveric specimens were separated into three donor age groups. These were allocated into three harvesting subgroups: group a, single pieces (cephalocaudal segments); group b, two laminated pieces of the same rib sutured together in anatomical position (laminated group); and group c, two pieces from the same rib reversed onto each other and sutured together (oppositional group). Photographs were examined and analyzed to determine the degree of cartilaginous warping. Decreased rates of warping were seen in commercially available, aseptically processed costal cartilage allografts procured from older cadavers. Warping was also decreased when oppositional suturing techniques were used as a way to address those intrinsic cartilage forces. (*Plast. Reconstr. Surg.* 146: 37e, 2020.)

Correction of aesthetic and functional nasal deformities presents a significant challenge for the rhinoplasty surgeon.[1] Cartilage is often added to the nasal osseocartilaginous framework to provide support, improve aesthetics, and maintain a patient nasal airway. Septal cartilage is commonly used as a primary source of cartilage. However, in cases where this is unavailable or insufficient, especially in revision rhinoplasty, rib is the best alternative donor site because of its abundant supply, harvest reliability, volume, and intrinsic strength.[2–4] Rib cartilage grafts can be produced with considerable versatility with respect to shape, length, and width, facilitating structural reconstruction of different types of functional and aesthetic requirements, namely, in the nasal dorsum, septum, tip, and/or alar rims.[2,4] Use of autologous costal cartilage grafts may be limited by donor-site morbidity, patient choice or refusal for additional operative sites, and requirement for increased operative time.[1]

The senior author's (R.J.R.) preference when septal cartilage is lacking is a commercially available costal cartilage allograft. Such allografts have "off-the-shelf" accessibility, are available in multiple sizes, are aseptically processed to meet U.S. Pharmacopeia (71) sterility, and are screened to minimize infectious risks. Allografts processed with pretreatment gamma irradiation (U.S. Food and Drug Administration approved) instead of terminal sterilization are commercialized as safe and more similar to autograft structure, possibly because of less radiation exposure. Previous studies have reported that higher doses of allograft irradiation lead to lower stiffness and greater resorption.[5] Benefits of allografts include biocompatible material with less likelihood of infection and extrusion compared with alloplastic implants, and no secondary surgical site with associated issues related to donor-site morbidity of autologous graft harvest. This allows for a reduction in operative time and surgical costs. The different sizes available can match surgeon preference and reduce trimming and graft waste.

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Unfortunately, costal cartilage is prone to warping because of its inherent characteristics related to interlocking stresses of the collagen scaffolding. Pioneers in rhinoplasty, such as Gillies, Gibson, Davis, Fry, Millard, Gunter, Daniel, and Rohrich, have described various costal cartilage harvesting and prefabrication techniques to decrease the intrinsic warping tendencies of rib cartilage.[3,4,6–19] The further popularized concept of balanced cross-sectional central costal cartilage harvest has assisted in decreasing the degree of warping observed but did not completely prevent it. The understanding of costal cartilage's physiologic features such as ossification with increasing age and the intrinsic forces leading to warping can be valuable for choosing the most appropriate types of cartilage specimens and suturing techniques to prevent warping.

During aging, changes in appearance and physical characteristics occur in costal cartilage microstructure, particularly dehydration of tissue, yellowish pigment deposits, calcification, and ossification.[20] Consequently, some studies report a decrease in costal cartilage flexibility over time, which becomes less pliable, yellowish, and inhomogeneous when cut.[20,21] Theoretically, partial cartilaginous ossification may increase strength and decrease warping tendency. Costal cartilage of relatively older patients with higher calcium content was associated with a reduced incidence of graft warping.[21] As described previously, redirecting the intrinsic stressor forces toward themselves by using oppositional suturing techniques may also help in prevention of warping.[3,4] The purpose of this study was to evaluate the effect of donor age, storage temperature, and orientation of a bilayered construct on the degree of warping of a commercialized fresh frozen costal cartilage allograft (MTF Biologics, Edison, N.J.) in vitro over time.

MATERIALS AND METHODS

A total of 140 costal cartilage cadaveric specimens were provided frozen by MTF Biologics and separated into three donor age groups: (1) older age (aged 50 years or older, $n = 32$); (2) middle age (36 to 50 years, $n = 60$); and (3) younger age (20 to 35 years, $n = 48$). Cartilage segments were sharply stripped of perichondrium and cut manually with a no. 10 blade by five plastic surgeons. Cartilage allografts were cut into sections of approximately 30 × 10 × 2 mm, consistent with the senior author's (R.J.R.) preferred dimensions and thickness. After preparation of allograft sections from each age group, they were allocated into three harvesting subgroups: group a, single pieces (cephalocaudal segments); group b, two laminated pieces of the same rib sutured together in anatomical position (laminated group); and group c, two pieces from the same rib reversed onto each other and sutured together (oppositional group). Laminated and oppositional pieces were sutured together by means of two simple interrupted stitches with 5-0 polydioxanone suture. The three harvesting subgroups were then stored in three ways: frozen (18°F; 0°C), refrigerated in saline at 35°F (1.6°C), and room temperature in saline (76°F; 24°C).

Warping Analysis

All cartilage was cut and packaged on day 0. Assessment of cartilage warping was performed on a weekly basis. All photographs were taken with a high-resolution camera on a 1-mm-grid background. Six pieces of cartilage from each subgroup of single pieces were selected randomly (1.a.α.; 1.a.β; 1.a.γ; 2.a.α.; 2.a.β; 2.a.γ; 3.a.α.; 3.a.β; 3.a.γ). These six individual pieces from each age group/storage subgroup were designated as the core (baseline) group and were studied at all evaluation points. A different and additional random sample of six single pieces from each age group/storage subgroup was added in each evaluation point. All cartilage samples from laminated and oppositional subgroups from each age group/storage subgroup were evaluated at every time point assessment. Photographic evaluations of cartilage warping were conducted every 2 weeks for 3 months.

Photographic Evaluation

Photographs were examined and analyzed to determine the degree of cartilaginous warping in a modified manner described by Foulad et al.[22,23] Digital images were uploaded into Adobe Photoshop CS5.1 (Adobe Systems, Inc., San Jose, Calif.). The "Magic Wand Tool" was used to isolate the convex surface of the cartilaginous segment. This convexity (i.e., warping was evaluated against the 1-mm-grid background to formulate percentage warping from the baseline image. These were performed at multiple points for each rib sample analyzed and averages were obtained. Statistical analysis was performed using IBM SPSS Version 19 (IBM Corp., Armonk, N.Y.). Comparisons were made between subgroups using two-sided paired t tests. One-way analysis of variance was used to compare change in warping over time. The level of statistical significance was set at $p < 0.05$.

RESULTS

A total of 186 segments of allograft costal cartilage specimens were obtained, with each measuring approximately 30 × 10 × 2 mm. Of these specimens, 45 were deemed unusable for the study because of either technical error in processing/cutting or atypical ossification or composition. The remaining 141 segments of allograft costal cartilage were divided into three age groups (young age, $n = 48$; middle age, $n = 60$; and older age, $n = 32$). and further divided into three groups: (1) cephalocaudal segments, (2) oppositional segments, and (3) laminated segments. Specimens from each of these groups were placed into three different temperature conditions: (1) frozen, (2) refrigerated, and (3) room temperature.

To determine the conditions (age, cartilage cut, temperature) and levels (cephalocaudal, oppositional, or laminated) that had an effect on cartilage warping, the 141 samples were analyzed using a three-way analysis of variance test (with planned post hoc pairwise comparisons using the Bonferroni correction). Samples from the three age groups were assigned randomly to three cartilage cut conditions (i.e., cephalocaudal, laminated, and oppositional) and three temperature conditions (i.e., refrigerated, frozen, and room temperature).

Table 1 presents the distribution of the cartilage samples. Given the small sample size per cell, it was unlikely that any two-way interactions or the three-way interaction would prove to be statistically significant; however, these interactions were considered in the analysis in addition to the three main effects, with the three main effects of primary interest for this study.

Table 2 presents the results of the three-way analysis of variance, including the main effects and interaction effects. Age group, cut, and temperature all had a significant main effect on warping of the cartilage. Based on the partial eta-squared values, age group and temperature could be considered very large effects, whereas the cut could be considered a medium to large effect. Specifically, the age group and temperature differences were very large, whereas the cut differences were moderately large. Although the overall analysis of variance results do not demonstrate where the differences among the three categories within the three independent variables lie, the pairwise comparisons within each independent variable demonstrates where the warping mean values differ.

Table 3 displays the post hoc pairwise comparison results for the age groups. The samples in the group aged 20 to 35 years had significantly higher rates of observed cartilage warping compared to the samples in the group aged 36 to 50 years and the group older than 50 years. However, the samples from the group aged 36 to 50 years did not differ statistically from the samples from the group older than 50 years, indicating that warping drops off in the samples obtained from middle-aged cartilage.

Table 4 displays the post hoc pairwise comparison results for the cartilage cut groups. The only group difference was between cephalocaudal and oppositional in that oppositional had lower warping compared to cephalocaudal. Laminated values were not different statistically from both oppositional and cephalocaudal samples.

Table 5 displays the post hoc pairwise comparison results for the temperature groups. The mean values for all three pairs were statistically different from one another, with room temperature stored cartilage having the highest warping values, refrigerated cartilage having medium warping values, and frozen cartilage having the lowest warping values.

The following conclusions can be made based on the data: (1) cartilage from individuals older than 36 years are less subject to warping than cartilage from individuals younger than 36 years, (2) oppositional orientation and to a lesser extent laminated orientation will produce lower warping levels compared with cephalocaudal cutting, and (3) frozen cartilage will produce the least amount of warping. In addition, although there was no interaction effect because of cell size limitations, it is possible that cutting frozen cartilage using the oppositional method from cadavers older than 50 years will produce the lowest levels of tissue warping (Fig. 1). Figure 1 demonstrates the average warping by the nine groups explored.

Table 1. Distribution of Cartilage Samples

	Age Group			
Cut and Temperature	20–35 Yr	36–50 Yr	>50 Yr	Total
Cephalocaudal				
Room temperature	6	8	3	17
Refrigerated	5	6	3	14
Frozen	5	6	4	15
Laminated				
Room temperature	6	8	3	17
Refrigerated	5	6	3	14
Frozen	5	6	4	15
Oppositional				
Room temperature	7	7	6	20
Refrigerated	5	6	2	13
Frozen	5	6	5	16
Total	49	59	33	141

Table 2. Three-Way Analysis of Variance Results*

Source	Type III SS	*df*	MS	F	*p*	Partial η^2
Age group	9.73	2	4.87	69.34	<0.001	0.55
Cut	0.87	2	0.43	6.19	<0.01	0.10
Temperature	5.94	2	2.97	42.36	<0.001	0.43
Age group × cut	0.25	4	0.06	0.88	0.48	0.03
Age group × temperature	0.36	4	0.09	0.13	0.28	0.04
Cut × temperature	0.32	4	0.08	1.14	0.34	0.04
Age group × cut × temperature	0.49	8	0.06	0.87	0.55	0.06
Error	8.00	114	0.07			
Corrected total	26.61	140				

SS, sum of squares; *df*, degrees of freedom; MS, mean squares; F, F ratio.
*Age group, cut, and temperature all had a significant main effect on warping of the cartilage.

Table 3. Post Hoc Age Pairwise Comparison Results

			Bonferroni Comparison *p*		
Age Group (Yr)	Mean	SEM	20–35 Yr	36–50 Yr	>50 Yr
20–35	1.32	0.04	n/a	<0.001	<0.001
36–50	0.80	0.04		n/a	0.21
>50	0.69	0.05			n/a

n/a, not applicable.

Table 4. Post Hoc Cut Pairwise Comparison Results

			Bonferroni Comparison *p*		
Cut	Mean	SEM	Cephalocaudal	Laminated	Oppositional
Cephalocaudal	1.04	0.04	n/a	0.31	<0.01
Laminated	0.94	0.04		n/a	0.20
Oppositional	0.83	0.04			n/a

n/a, not applicable.

Table 5. Post Hoc Temperature Pairwise Comparison Results

			Bonferroni Comparison *p*		
Temperature	Mean	SEM	Room Temperature	Refrigerated	Frozen
Room temperature	1.20	0.04	n/a	<0.001	<0.001
Refrigerated	0.91	0.04		n/a	<0.001
Frozen	0.69	0.04			n/a

n/a, not applicable.

DISCUSSION

In the present study, costal cartilage procured from older cadavers was associated with less warping. These results are consistent with those of previous literature. While looking at nasal augmentation with costal cartilage, Balaji found a statistically significant association between cartilage age and warping tendency. Costal cartilage from relatively older individuals was found to display characteristics of increased calcification and showed less warping during the study period.[21] The effect of calcification on the structural mechanics of costal cartilage has also been previously studied and shown to result in increased cartilage stiffness and potential for decreased resorption, both of which are highly desirable qualities for cartilage grafts in modern rhinoplasty.[24]

The other feature of costal cartilage that has repeatedly been found responsible for warping is that of intrinsic forces inherent in the harvested cartilage. Several harvesting and prefabrication techniques have been described to decrease the warping tendencies of rib cartilage, including the concept of balanced cross-sectional central costal cartilage harvest and oppositional suturing techniques. In our study, when the latter suturing technique was used, a decreased level of warping was noted.[2–4,21,23]

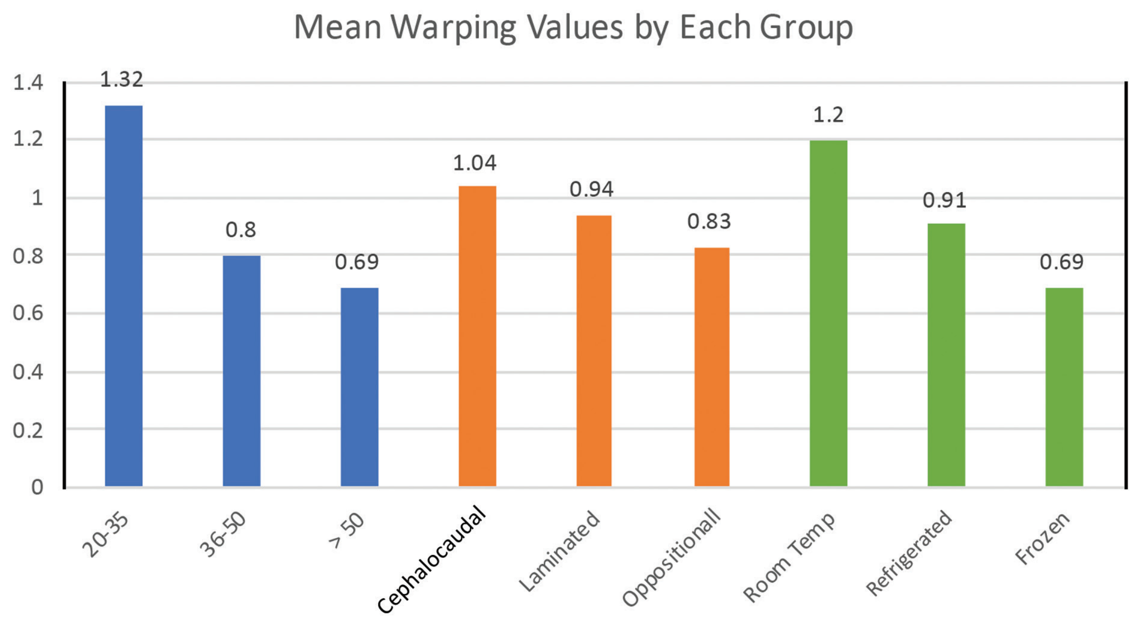

Fig. 1. Average warping for each of the nine groups explored.

When controlling for age and specimen lamination/suturing, it became clear that the trend remained unchanged between the specimens less prone to warping (older) compared to the specimens more prone to warping (younger). However, we did find a statistically significant difference in the overall degree of warping between freezer versus room temperature conditions. Our data demonstrate that in a freezer setting, there is less warping than in a room temperature setting. However, the clinical relevance of these data may not be useful, as these materials are implanted at body temperature.

Adams et al. found that the rate of warping in irradiated versus nonirradiated rib cartilage was comparable, whereas resorption rates were indeed variable.[1] Other studies report that higher doses of costal cartilage allograft irradiation have been associated with decreased graft stiffness and greater resorption.[5] The commercially available costal cartilage allografts used in this study are aseptically processed, with no terminal sterilization. In the senior author's experience, these aseptically processed allografts are significantly more predictable in the long term.

There are several limitations to the present study. Concentric and balanced carving of costal cartilage has been shown to play an important role in decreasing rate of warping.[2–4,21,23] Although precise dimension and thickness were strived for when cutting cartilage, there is an inherent imprecision when doing this by hand. This, however, might be more representative of how cartilage is treated in the operating room. Another possible limitation relates to how the cut cartilage was stored. Freely floating in saline solution (in vitro) does not expose the cartilage graft to the physiologic effects that might occur in the surgical wound bed. Fibrovascular ingrowth from surrounding soft tissue has the potential to alter the tendency of graft warping and reabsorption.

CONCLUSIONS

Decreased rates of warping were seen in commercially available, aseptically processed costal cartilage allografts procured from older cadavers. This is likely attributable to a higher calcification content than is seen in younger, whiter cartilage grafts. Warping was also decreased when oppositional suturing techniques were used as a way to address those intrinsic cartilage forces. Storage temperatures did impact warping in our study, with colder temperatures correlated to less warping, although the overall trend for age and suturing orientation remained consistent regardless of temperature category. Future studies are needed to explore how costal cartilage age affects resorption.

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