Study of Rabbit Septal Cartilage Grafts Placed on the Nasal Dorsum

Márcia Maria Ale de Souza, MD; Luiz Carlos Gregório, MD; Ricardo Sesso, MD; Soraía Ale Souza, MD; Flavio Settanni, MD

Objective: To compare the reabsorption characteristics of fresh septal cartilage autografts, preserved homografts, and preserved autografts in the nasal dorsum of rabbits.

Methods: Rabbit nasal dorsum cartilage grafts were placed in 3 groups. The first group used fresh autologous cartilage; the second group, alcohol-preserved homologous cartilage; and the third group, alcohol-preserved autologous cartilage. Each rabbit received 2 grafts, one crushed and another noncrushed. After 16 weeks, the grafts were removed for analysis.

Results: No graft calcification occurred in any group. Chondrogenesis was observed in all groups. The fresh autograft group had the best results in the evaluation of the area of graft recovered and chondrocyte viability. The preserved autologous and homologous grafts did not differ in relation to any of the variables analyzed. Crushed grafts had inferior results in the area of graft recovered and chondrocyte viability compared with the noncrushed forms. No significant difference among the 3 groups was noted in the thickness of the fibrous capsule that developed around the graft.

Conclusions: The fresh cartilage autograft was superior to the crushed and uncrushed preserved homografts and autografts; both types of preserved grafts had equivalent histological results. The uncrushed forms were superior to the crushed forms.

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The role of grafts and implants in aesthetic and reconstructive surgery of the nose has evolved considerably during the past several decades. The search for the ideal material has led to an exponential growth in research and development of different alloplasts. However, their use in the nose has been associated with unacceptably high rates of extrusion, infection, and rejection.1-3

Autogenous cartilage is considered to be ideal for all types of grafting in nasal surgery. The material survives as living tissue, is seldom resorbed, and does not stimulate an immune response, so problems with rejection, infection, and extrusion are rarely encountered with these grafts.2,4

The use of cartilage grafts is one of the most important procedures in reconstructive nasal surgery; however, many questions remain unanswered, even regarding the use of autologous cartilage. According to Guyuron and Friedman,5 preserved autologous cartilage remains clinically similar to fresh autologous cartilage. Some authors5-9 consider the best option to be the use of homologous material when autologous cartilage is not available in the needed amount.

The literature contains limited and contradictory information about the viability of crushed cartilage and the predictability of clinical outcomes with this material.3 In addition, we have not found quantitative studies of preserved autografts and homografts in the literature that could support their use. The aim of this experimental study was to compare fresh autologous cartilage, considered the criterion standard material, with preserved homografts and autografts in crushed and uncrushed forms and placed in the nasal dorsa of rabbits.

Methods

The adult rabbit is a well-established model for research about cartilage grafting because it has septal cartilage similar to human cartilage10 and is an established recipient site model for nasal dorsal implants.

We selected 120 days as our study end point to approximate the equivalent of more than 1 year in human life. We used 63 New Zealand white male rabbits (Oryctolagus cuniculus), aged approximately 1 year and weighing 2.75 to 3.50 kg. All procedures were performed under the animal care guidelines of the Committee on Animal Care institutional review board.

Sixty animals were randomly allocated into 3 groups of 20 and identified with a number on the right ear. The first group was given fresh autologous cartilage (FAC); the second group, alcohol-preserved homologous cartilage (PHC); and the third group, alcohol-preserved autologous cartilage (APC).
and the third group, alcohol-preserved autologous cartilage (PAC). Three animals were used to standardize the best technique to dissect the graft and to define the size of the septal graft that could be obtained. We chose to remove one 15 × 10-mm fragment and, subsequently, two 6 × 6-mm fragments as a standard graft measure.

Crushing was performed using a cartilage crusher (Cottle model 523900; Karl Storz GmbH & Co, Tutlingen, Germany). The grade of crushing was defined as moderate (ie, moderate force was used to soften the surface without reducing the elastic strength of the cartilage). We standardized the technique for cartilage crushing with 2 taps, always performed by the same researcher (M.M.A.S.) to avoid variations in the procedure. We also standardized the placement of the uncrushed septal cartilage fragment on the left side of the nasal dorsum and the crushed fragment on the right side.

Each rabbit’s nose was depilated under general anesthesia, then steriley prepared and draped. For the septum resection, the approach described by Gubisch et al11 was used. This involved folding back the alar ridge of the nose to obtain exposure of the septum. After hemitransfixion incisions, bilateral mucoperichondrial flaps were elevated and a 15 × 10-mm rectangle of cartilage (free of perichondrium) was removed. In the FAC group, the recipient site was prepared in the same surgical procedure as previously described by Maas et al12; a 1.0-cm anterior incision through the skin and subcutaneous tissues was made on the dorsum of the nose. A subcutaneous pocket extending 4 cm in the direction of the cranium was formed directly over the bony dorsum, superficial to the periosteum. Before placement of the 2 cartilage grafts, each separated by 0.5 cm in the pocket, they were measured with a surgical compass to obtain two 6 × 6-mm fragments. One of them was crushed before placement. The grafts were well secured with 6.0 nylon sutures. The incisions were closed in 1 layer with a 4.0 nylon suture.

In the PHC and PAC groups, 1 month before the placement of the septal graft on the nasal dorsum, the graft was removed (using the same septal approach as that used for the FAC group) and preserved in 70% alcohol. One month later, with the rabbit under general anesthesia, and using the same recipient site approach as that used for the FAC group, the graft was washed with isotonic sodium chloride solution and cut in 2 fragments as in the FAC group, and 1 fragment was crushed. In the PAC group, each animal received its own preserved septal cartilage. In the PHC group, the fragments were placed in the nasal dorsum of an animal different from the donor. During 16 weeks, no animal had signs of wound infection, wound dehiscence, flap necrosis, or graft extrusion. At the end of this period, the rabbits underwent anesthesia, the dorsum was reopened, and the grafts were removed along with the dense conjunctive tissue deposited around them. After removal, the length and width of the fragments were measured.

HISTOLOGICAL STUDY

Qualitative Studies

Postimplantation specimens were prepared for serial section and staining by hematoxylin-eosin and toluidine blue. Histological sections were evaluated with the use of an optic microscope (Labophot; Nikon Corporation, Tokyo, Japan), and the degrees of calcification, ossification, and neochondrogenesis were analyzed.

Quantitative Studies

The hematoxylin-eosin–stained sections were used to evaluate chondrocyte viability and the status of the chondroid tissue. The toluidine blue–stained sections were used to evaluate chondroid tissue matrix metachromasia and chondrocyte viability. Cartilage graft viability was based on the state of the chondroid matrix, specifically loss of matrix metachromasia and loss of chondrocyte nuclei from the lacunae. The absence of matrix metachromasia and chondrocyte nuclei was considered to indicate nonviable chondroid tissue. A morphometric analysis was performed to determine the percentage of viable chondrocytes in the grafts and the measurement of the thickness of conjunctive tissue. For each specimen, the percentage of viable chondrocytes was calculated using a device with 25 points per field, analyzing 12 microscopic fields (original magnification ×400) for a total of 300 points for each cartilage segment13 (Figure 1). The thickness of the conjunctive tissue around the graft was measured at 12 points in 3 histological sections (Figure 2). The average value of all measures was used as the result.

STATISTICAL ANALYSIS

We compared the continuous variables with normal distributions among the FAC, PHC, and PAC groups with 1-way analysis of variance. We used the Tukey test to assess the 2 × 2 differences between the groups. For continuous variables with nonparametric distribution, we performed the comparisons using the Kruskal-Wallis test. The comparison of continuous variables between crushed and uncrushed specimens within each study group was assessed with the Wilcoxon signed rank test. Statistical tests were 2 sided, and statistical significance was set at P < .05.
RESULTS

For the noncrushed grafts, the mean area of graft recovered was significantly larger in the FAC group than in the PHC (P < .001) or the PAC group (P < .001). The latter 2 groups had similar mean areas. The corresponding analysis for the crushed grafts revealed results similar to those for the noncrushed grafts. The FAC group showed a significantly larger mean area than the other 2 groups, and the PHC and PAC groups had similar results. Within the FAC group, the area of graft recovered was significantly larger when we used the noncrushed vs the crushed form (P < .001). Superior results with the noncrushed forms were similarly observed in the PHC and PAC groups. Overall (including the crushed and noncrushed specimens), the best results were obtained with noncrushed grafts in the FAC group. Nevertheless, the mean area of graft recovered using crushed FAC was significantly smaller than that obtained with uncrushed PHC (P < .001) or PAC (P < .001) (Table). The mean percentage of area of recovered grafts in relation to the area of implanted uncrushed specimens for the FAC, PHC, and PAC groups was 86.3%, 68.5%, and 71.4%, respectively (P < .001); the corresponding figures for the crushed forms were 44.9%, 31.9%, and 36.1% (P < .001).

For the analysis of viable chondrocytes, the results of the comparisons within and among the FAC, PHC, and PAC groups were exactly the same as those reported for the area of graft recovered (Table and Figures 3, 4, 5, and 6).

The mean thickness of the capsule of dense connective tissue was similar among the crushed forms in the FAC, PHC, and PAC groups; the corresponding comparisons among the 3 groups when we used the noncrushed forms were also equivalent. Within each group (FAC, PHC, and PAC), the thickness of the capsule was significantly greater (P < .01) using crushed vs noncrushed grafts (Table).

COMMENT

Cartilage grafting has been used extensively to correct nasal framework deformities. The septum is often the preferred source of autogenous cartilage; however, it may be present in insufficient quantity for the reconstructions. Another donor site may be necessary, but this is limited by donor-site morbidity and increased operative time. In revisional surgeries, some authors advocate the use of a preserved autologous cartilage bank; another alternative is the use of a preserved costal cartilage homograft, as suggested by Velidedeoglu et al.9

One of the most important issues regarding cartilage grafts in the nose is the degree of reabsorption. In a study using radiographic analysis, Donald and Coy found minimal reabsorption in irradiated cartilage homografts implanted in the sheep facial skeleton. In the literature, there are conflicting data about the results with preserved autografts and homografts.7,14-16

In accordance with Keskin et al,7 who performed an experimental study with homografts in tracheal stenosis, we also used 70% alcohol as the preservation solution. Adlington et al reported that different modes of preparation of mouse costal cartilage homografts (irradiation, formalin, glutaraldehyde, and alcohol) did not influence observed fibrosis and reabsorption.

The literature contains limited and contradictory information about the viability of crushed cartilage and the predictability of its clinical outcomes.18-20 Most sur-

Table. Histological Findings 16 Weeks After Grafting in the Study Groups

<table>
<thead>
<tr>
<th>Study Groups, Mean (SD)</th>
<th>FAC</th>
<th>PHC</th>
<th>PAC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Noncrushed</strong> (n = 20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area of the graft recovered, mm</td>
<td>31.4 (4.2)</td>
<td>24.7 (4.7)</td>
<td>25.7 (4.8)</td>
</tr>
<tr>
<td>Viable chondrocytes, %</td>
<td>44.4 (2.1)</td>
<td>22.9 (5.0)</td>
<td>23.5 (4.0)</td>
</tr>
<tr>
<td>Thickness of the capsule of dense connective tissue, µm</td>
<td>25.1 (10.1)</td>
<td>32.0 (9.7)</td>
<td>31.9 (9.3)</td>
</tr>
<tr>
<td><strong>Crushed</strong> (n = 20)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area of the graft recovered, mm</td>
<td>16.2 (3.4)</td>
<td>11.5 (3.9)</td>
<td>13.0 (3.8)</td>
</tr>
<tr>
<td>Viable chondrocytes, %</td>
<td>10.6 (0.9)</td>
<td>7.1 (1.8)</td>
<td>7.5 (1.5)</td>
</tr>
<tr>
<td>Thickness of the capsule of dense connective tissue, µm</td>
<td>36.4 (10.9)</td>
<td>40.6 (12.0)</td>
<td>40.5 (11.8)</td>
</tr>
</tbody>
</table>

**Abbreviations:** FAC, fresh autologous cartilage; PAC, alcohol-preserved autologous cartilage; PHC, alcohol-preserved homologous cartilage.

a P < .001 compared with the noncrushed PHC and PAC groups.
b P < .001 compared with the noncrushed PHC and PAC groups.
c P < .001 compared with the crushed PHC and PAC groups.
d P = .007 compared with the noncrushed PAC group.

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geons are hesitant to use this material for contour restoration in rhinoplasty. In our study, noncrushed fresh autografts yielded the greatest mean area of recovered graft in relation to the implanted graft (86.3%), followed by the noncrushed PAC (71.4%) and noncrushed PHC (68.5%). Using the crushed forms, the area of recovered grafts was considerably reduced (FAC, 44.9%; PAC, 36.1%; and PHC, 31.9%). The recovered area of the crushed form of FAC was significantly smaller than those of the noncrushed forms of PAC and PHC. These findings support the suggestion of Brown et al, who proposed that this decrease was caused by the destruction of the polysaccharide matrix, thus exposing antigens, with a resulting increased reabsorption. Other authors have also found worse results with crushed cartilage, but have not proposed any explanation. In a study with rabbit cartilage, Rudderman et al obtained a greater percentage of recovered graft volumes, including 94.5% for noncrushed FAC, 69.7% for crushed FAC, 91.3% for noncrushed PAC, and 74.2% for crushed PAC. Our results do not confirm those of Keskin et al, who found that noncrushed FAC and PHC yielded similar recovered graft volumes when ear grafts were used for tracheal stenosis in rabbits.

We found no calcification in any implant specimen. In another study with rabbit dorsal cartilage autografts, minimal calcification was reported using grafts from the septum, auricle, and rib.

Our results regarding the percentage of viable chondrocytes were similar to those of the area of recovered graft; the noncrushed forms were superior to the crushed forms, with the FAC group showing the best results. In a rabbit model of fresh crushed cartilage graft, Rudderman et al documented a volume retention after 90 days of 70% and a chondrocyte viability rate of 70%; they concluded that crushed cartilage could be used to attain aesthetic goals, recommending overcorrection to achieve optimal final results. On the other hand, in our crushed FAC group, we obtained only 11% viable chondrocytes. In a study using mildly crushed human septal cartilage (similar to our degree of morselizing), Bujía reported 30% chondrocyte viability. However, unlike our study, Bujía examined the viability of cartilage using cell cultures only once, 1 to 2 days after crushing. In an experimental work in rabbits, Breadon et al re-
ported that all uncushrashed cartilage grafts remained viable. Guyuron and Friedman\textsuperscript{2} revealed a surgical success rate of 87% with fresh crushed grafts. Yilmaz et al\textsuperscript{21} found that crushed cartilage grafts in a rabbit model were viable after extensive crushing. In contrast, we observed that, irrespective of the group (FAC, PAC, or PHC), the chondrocyte viability decreased remarkably in the crushed forms. In fact, rabbit experiments by Verwoerd-Verhoef et al\textsuperscript{22} showed that crushed cartilage grafts led to extensive necrosis and that only 10% to 30% of cells survived the process, depending on the crushing technique used. We believe that the cartilage crushing led to diminished viability and partial transformation to connective tissue, reducing graft volume, as already suggested by Huizing.\textsuperscript{18} Therefore, our findings suggest that crushed cartilage may be only used for filling small nasal defects, with a sligh overcorrection during placement of crushed cartilage grafts.

There are some difficulties when our results are compared with those in the literature: most studies used groups with FAC and PHC, not PAC, and they assessed the grafts after a shorter time since implantation.\textsuperscript{2,12,20} Although fresh autogenous septal cartilage is the first-line graft choice for rhinoplasty when this material is available, most of the animal studies summarized herein involved auricular cartilage.\textsuperscript{19-23} In addition, the severity of crushing varies among the studies. This lack of standardization of crushing intensity may be a major reason for the conflicting results noted among the studies. In a recent study in rabbits, Cakmak et al\textsuperscript{22} assessed the influence of crushing level and found that, as crushing intensity increased, more cartilage cells were irreversibly damaged, more cartilage tissue was transformed to connective tissue, and chondrocyte proliferation at the periphery decreased, leading to a reduction in graft volume.

When we evaluated the thickness of the capsule of dense connective tissue, there was no significant difference among the FAC, PHC, and PAC groups. However, the crushed form had a thicker connective tissue capu-"