Viability of Crushed and Diced Cartilage Grafts

A Study in Rabbits

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Objective: To investigate the effects of dicing and different degrees of crushing on cartilage graft viability and outcome in rhinoplasty.

Methods: Cartilage was harvested from both ears of 29 rabbits. For each animal, 6 cartilage pieces were prepared as follows and inserted into the paraspinal subcutaneous tissue: (1) left intact, (2) diced to approximately 1 × 1-mm pieces and then wrapped in oxidized regenerated cellulose, (3) slightly crushed, (4) moderately crushed, (5) significantly crushed, and (6) severely crushed. Animals were killed at 2, 5, and 10 months, and graft specimens were microscopically examined.

Results: As crushing intensity rose, cartilage viability decreased and more cartilage tissue was transformed to connective tissue. The intact and slightly crushed grafts showed significant chondrocyte proliferation. This decreased as crushing intensity increased, and the severely crushed and diced cellulose-wrapped grafts exhibited almost no peripheral chondrocyte proliferation.

Conclusions: Slight crushing of a cartilage graft can produce outstanding graft material that forms softer nasal contours and fills defects well. However, severe crushing of cartilage grafts results in extensive necrosis and eventual reduction in graft volume. The use of oxidized regenerated cellulose to wrap diced cartilage grafts also tends to reduce clinical predictability owing to negative effects on cartilage viability and regeneration.

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Dorsal irregularities of the nose can become apparent after rhinoplasty, which remains a major concern for surgeons. Such problems tend to become more obvious after edema subsides and, if the skin is thin, may be even more prominent years after surgery. Various graft materials, including cartilage, resected nasal hump tissue, dermal grafts, temporoparietal fascia grafts, and alloplastic materials, have been used to prevent the "operated-on look" and to mask dorsal irregularities after rhinoplasty. Grafts may either resorb or become visible in the late postoperative period. Autogenous cartilage is considered ideal for all types of grafting in nasal surgery. This 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.

It has been suggested that crushed and diced autogenous cartilage grafts may be used to conceal dorsal irregularities and to help achieve a smoother dorsal surface after rhinoplasty. However, the literature contains limited and contradictory information about the viability of crushed cartilage and the predictability of clinical outcome. Also, it is not yet clear whether the intensity of crushing affects cartilage viability and/or long-term outcome. Because of conflicting information and the unpredictable behavior of crushed and diced cartilage grafts, most surgeons are hesitant to use these materials for contour restoration in rhinoplasty.

The main aim of this study was to investigate the effects of dicing and different degrees of crushing on autogenous cartilage graft viability and outcome in rhinoplasty. We also compared outcomes with crushed cartilage grafts with results from diced cartilage wrapped in cellulose and noncrushed, solid-carved cartilage grafts.

METHODS

A total of 33 New Zealand white rabbits were acquired for the experiment, and data from 29 animals that survived all procedures were used. Four rabbits died before the end of the study, and these were excluded. The 29 animals were aged 8 months to 1 year and weighed 2 to 3 kg. The Ethics Committee of Baskent University approved the study, and all procedures...
Figure 1. Harvesting cartilage from a rabbit's ear.

Figure 2. One set of 6 grafts (left to right): intact, slightly crushed, moderately crushed, significantly crushed, severely crushed, and diced. The inserted image shows an intact cartilage graft and a diced cartilage graft on a piece of oxidized regenerated cellulose.

complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.11

Each rabbit was given general anesthesia with an intramuscular injection of 50 mg/kg of ketamine hydrochloride and 5 mg/kg of xylazine hydrochloride. Cartilage grafts were harvested from both ears (Figure 1). The dorsal and ventral perichondrial layers were removed from each piece of resected tissue, and the cartilage was sectioned into 1-× 1-cm pieces. Crushing was performed using a Cottle cartilage crusher (model 523900; Karl Storz GmbH & Co, Tutlingen, Germany), and 6 cartilage pieces from each rabbit were prepared as follows: (1) left intact, (2) diced and then wrapped in oxidized regenerated cellulose (Surgicel; Johnson & Johnson Gateway, Piscataway, NJ), (3) slightly crushed, (4) moderately crushed, (5) significantly crushed, and (6) severely crushed (Figure 2). In the second group, a number 15 blade was used to dice each graft into pieces of approximately 1 × 1 mm, and these pieces were then wrapped in a single piece of oxidized regenerated cellulose. The different grades of crushing were defined as follows: slightly crushed, 1 hit of moderate force to soften the surface without reducing cartilage elastic strength; moderately crushed, 2 moderate-force hits to soften the surface and also reduce elastic strength; significantly crushed, 3 to 4 moderate-force hits, enough to cause the graft to bend with gravity; and severely crushed, 5 to 6 forceful hits to totally destroy the integrity of the cartilage.

During the same anesthesia session, the skin on the rabbit's back was shaved and surgically prepared. Then 6 incisions approximately 1.5 cm long were made in the skin of the paraspinal region, and a small subcutaneous pocket was created at each site. The 6 cartilage preparations were inserted into the 6 subcutaneous pockets of the same animal (Figure 3A), and the incisions were sutured with 4-0 plain catgut.

There were no problems with infection, extrusion, seroma formation, or hematoma formation at the recipient sites in the postoperative period. Groups of animals were killed with lethal doses (150 mg/kg) of thiopental sodium at 2 months (n = 11), 5 months (n = 13), and 10 months (n = 5) after surgery. The recipient sites were excised immediately after each animal was killed (Figure 3B), and the specimens were fixed in 10% formaldehyde and then embedded in paraffin.

Five-micrometer-thick sections of each specimen were prepared with hematoxylin-eosin, toluidine blue, and Masson trichrome stain and were examined under light microscopy. The hematoxylin-eosin–stained sections were used to evaluate chondrocyte viability and the status of the chondroid tissue. Sections prepared with Masson trichrome stain were used to assess connective tissue, and the toluidine blue–stained sections were used to evaluate chondroid tissue matrix metachromasia and chondrocyte viability.

In the descritption of crush, the following notation was used. Intact: 100% intact cartilage, no peripheral chondrocyte proliferation or bone metaplasia; lodged (with gravity), 1 to 2 moderate-force hits, enough to cause the graft to flatten; elastic strength; moderately crushed, 2 moderate-force hits, enough to cause the graft to bend with gravity; and severely crushed, 5 to 6 forceful hits to totally destroy the integrity of the cartilage.

Cartilage graft viability was determined based on the state of the chondroid matrix, specifically loss of matrix metachromasia and loss of chondrocyte nuclei from the lacunae. Absence of matrix metachromasia and chondrocyte nuclei was considered to indicate nonviable chondroid tissue. The level of viability for each specimen was expressed as a percentage of the total tissue present. Chondrocyte proliferation and transformation to connective tissue were assessed semiquantitatively.

None of the groups showed any change in the proportion of surviving cartilage during the 10 months. The other histologic findings at the 3 stages were also similar in each group. At all 3 stages, higher intensity of crushing was associated with more irreversibly damaged cartilage and more transformation of cartilage tissue to connective tissue.

The mean proportion of viable cartilage observed in the specimens was 10% for diced cellulose-wrapped grafts, 10% for severely crushed grafts, 30% for significantly crushed grafts, 50% for moderately crushed grafts, 70% for slightly crushed grafts, and 90% for intact cartilage.

The intact grafts showed significant chondrocyte proliferation at the periphery (Figure 4). The slightly crushed grafts exhibited prominent peripheral chondrocyte proliferation and also showed bone metaplasia and minimal fibrous-collagenous tissue (Figure 5). As the

RESULTS

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intensity of crushing increased from this level, we noted less chondrocyte proliferation, lower graft viability, and increased amounts of fibrous-collagenous tissue (moderate crushing) (Figure 6). The severely crushed and diced cellulose-wrapped grafts exhibited massive destruction of the chondroid matrix, extensive loss of viability, and almost no chondrocyte proliferation at the periphery (Figure 7 and Figure 8). Six (21%) of the intact and 14 (48%) of the diced cellulose-wrapped grafts showed bone metaplasia, but this finding was much more frequent in the crushed grafts (24 [83%] slightly crushed, 22 [76%] moderately crushed, 22 [76%] significantly crushed, and 20 [69%] severely crushed specimens) (Figure 9).

COMMENT

Nasal hump resection converts the smooth, fused nasal dorsum into its components. This may expose sharp, irregular edges of bone and cartilage and/or a twisted septum and may also result in open roof deformity and pinched middle third of the nasal dorsum due to the removal of the spreader effect of the hump.1,2 As explained herein, these dorsal irregularities often become apparent after edema subsides. In patients who have thin skin on the nose, the edges of the underlying osseocartilaginous skeleton tend to become more prominent as years progress after the operation.2,3,6 Placement of a dorsal graft can prevent the skin from coming into direct contact with the underlying irregular nasal framework. Several types of grafts have been suggested for this purpose and to prevent the “operated-on look”; however, none is entirely satisfactory. Different graft materials have individual disadvantages depending on their source and also carry risks of later visibility, distortion, and resorption.

Some authors have stated that autogenous soft tissue grafts, such as temporalis fascia3 and dermal grafts,4 are satisfactory for covering underlying dorsal irregularities. Others have claimed that the volume of these grafts may decrease after several years.2,4 Alloplastic materials, such as gelatin film,6 polyglactin 910,7 and expanded polytetrafluoroethylene,8 have also been advocated for this purpose. However, absorbable synthetics do not last long,
and nonabsorbable synthetics have the highest rates of infection and extrusion of all graft materials.\(^2,10\) The latter should be removed if infection develops.

In contrast, autogenous cartilage is widely accepted as ideal for all types of grafting. This material survives as living tissue and seldom undergoes resorption.\(^9-11\) Since it does not stimulate an immune response, graft rejection, infection, and extrusion are rare.\(^10\) However, even when solid-carved pieces of this material are carefully shaved before placement, if the patient has thin skin, the edges of the graft may become apparent and cause dorsal contour irregularity. McKinney et al\(^12\) popularized the use of cartilaginous autogenous thin septal grafts that measured 35 × 8 mm and less than 1 mm thick to mask residual deformities. The main issue with this method is that it is not always possible to acquire this large an intact cartilage graft, especially in revision cases. Alternatives such as diced or crushed autogenous cartilage grafts may be effective for smoothing out dorsal irregularities and filling defects.

The diced cartilage graft is a material chopped into multifaceted particles, generally 0.5 to 3 mm in diameter. Its use was first introduced by Peer\(^13\) in 1943 and has been popularized by Erol.\(^14\) The study by Peer showed that diced rib cartilage grafts remained viable after they were stored beneath the skin in 7 patients. Histologic examination of these tissues revealed no degenerative changes of cartilage cells and no invasion or resorption of the hyaline matrix. The same report also noted that the spaces between the small cubes of cartilage in the graft were initially occupied by blood and later by ingrowing connective tissue. The author suggested that this filling of the spaces between the cartilage pieces with connective tissue added to the overall bulk of the diced graft and prevented contracture of the cartilage mass.

Diced cartilage is acknowledged to be excellent for filling purposes, but it may also be difficult to introduce and retain in tissue. Some investigators\(^15\) have implanted diced cartilage into prepared tissue pockets by syringing the graft material through small incisions; others\(^14\) have wrapped such grafts in oxidized regenerated cellulose before placement. Erol recommended slight overcorrection when placing such wrapped grafts. In an experimental study in rabbits, Yilmaz et al\(^16\) examined the viability of diced cartilage grafts and the effect of oxidized regenerated cellulose by comparing results in 3 groups: intact cartilage grafts, crushed cartilage grafts, and diced cartilage grafts, with subgroups of each being bare and wrapped in oxidized regenerated cellulose. The cartilage grafts wrapped with cellulose were placed in the subcutaneous tissue on one side of the animals, and the bare grafts were placed on the other side as controls. The authors found that the cartilage grafts in all 3 groups were viable. The cellulose-wrapped grafts showed markedly higher collagen content than the bare cartilage grafts. The bare grafts showed significant chondrocyte proliferation, whereas the cellulose-wrapped ones showed no evidence of proliferation.

Our findings are in line with those of Yilmaz et al.\(^16\) The intact and slightly crushed grafts showed significant new cartilage formation at the periphery, whereas the diced cellulose-wrapped grafts exhibited almost no peripheral growth activity. Although the mean proportion of viable graft material in the intact cartilage group was 90%, the mean viability in the diced cellulose-wrapped group was 10%. These results show that oxidized regenerated cellulose not only inhibits cartilage proliferation but also decreases cartilage viability. Owing to these negative effects, wrapping diced cartilage with oxidized regenerated cellulose may reduce long-term graft predictability in the clinical setting. These findings may explain why surgeons in clinical trials have advocated overcorrection when using diced cellulose-wrapped cartilage.

The crushed cartilage graft is an option that various authors\(^15,17,18\) have recommended for concealing dorsal irregularities. However, there is limited information about the viability of crushed cartilage and the predictability of clinical outcome with these grafts. The results in the lit-
erature are conflicting. Huising17 found that crushing the cartilage caused diminished viability and partial transformation to connective tissue, thus slightly reducing graft volume. As a result, the author recommended slight overcorrection during placement of crushed grafts. Similarly, on the basis of a clinical study, Rudderman et al19 stated that crushed cartilage could be used with a fair degree of predictability but recommended overcorrection to achieve optimal final results. In experimental work in rabbits by Breadon et al,18 all crushed and uncrushed cartilage graft specimens remained viable. The same study demonstrated that crushed cartilage not only remained viable but also induced formation of new bone and cartilage around and within the graft. In addition, the investigators observed active erythropoietic marrow in many of the specimens. They speculated that graft resorption would be balanced by cartilage and bone genesis around and within the graft and that postoperative cosmesis would be close to the appearance after surgery. Thus, Breadon and colleagues identified crushed autogenous cartilage as the preferred material for filling small nasal-facial defects.

The study by Yilmaz et al16 also showed that crushed cartilage grafts were viable, even after extensive crushing. Rudderman et al19 documented a retained volume of 70% and viability of approximately 70% to 90% with fresh crushed cartilage grafts in rabbits. Similarly, Guyuron and Friedman20 reported a surgical success rate of 87.5% with fresh crushed cartilage grafts in a clinical evaluation. In contrast, using an in vitro technique, Bujia21 found that crushing and cutting cartilage graft material led to partial necrosis of the graft. Concerning proportional viability, the study by Bujia revealed figures of 10% for severely crushed cartilage, 30% for slightly crushed material, 85% for diced grafts, and 90% for intact cartilage grafts. The author found that more cartilage cells were irreversibly damaged as the severity of crushing increased and that these necrotic portions were eliminated and replaced by fibrous tissue. An experimental study in rabbits by Verwoerd-Verhoef et al22 demonstrated only 10% to 30% cell survival after crushing of cartilage grafts, depending on the crushing technique used. These investigators also observed that crushing led to extensive necrosis and that necrotic tissue was eliminated and replaced by new cartilage formed from surviving chondrocytes. In light of the conflicting evidence presented herein, it has not been possible to make a conclusion about the viability or clinical predictability of crushed cartilage grafts.

In our study, we assessed the influence of crushing level in an attempt to resolve some of the conflicts in the literature. We found that as crushing intensity increased, more cartilage cells were irreversibly damaged and more cartilage tissue was transformed to connective tissue. In line with this, the mean proportion of viable cartilage decreased as the degree of crushing increased (70% for slightly crushed grafts, 50% for moderately crushed, 30% for significantly crushed, and 10% for severely crushed), whereas the viability result for intact cartilage grafts was 90%. We also noted that chondrocyte proliferation at the periphery decreased as the degree of crushing increased and was almost completely absent in the severely crushed grafts. In summary, our results show that graft cartilage viability, cartilage proliferation, and predictability of clinical outcome all decrease as crushing intensity rises. These are also factors in graft failure, which may explain why surgeons have recommended some degree of overcorrection when using crushed cartilage.
cartilage material in the nasal dorsum. In contrast to the outcome with more extensive crushing, our results show that slight crushing of cartilage induces variable amounts of chondrocyte proliferation and metaplastic bone formation while preserving viability. Formation of new cartilage and bone may help soften the contours of the nasal dorsum and balance any resorption that occurs. In our opinion, slight crushing of a cartilage graft produces outstanding graft material that is effective for masking dorsal irregularities, filling defects, and creating a smoother surface.

The degree of crushing is an important factor in cartilage viability and clinical predictability of autogenous cartilage grafts. Slight crushing causes variable amounts of chondrocyte proliferation and metaplastic bone formation while preserving viability. This level of crushing can produce outstanding graft material that allows the surgeon to achieve softer nasal contours and effectively conceal dorsal irregularities. However, as the severity of crushing increases, cartilage viability, chondrocyte proliferation, and predictability of clinical outcome all decrease. The use of oxidized regenerated cellulose to wrap diced cartilage grafts also tends to reduce clinical predictability owing to negative effects on cartilage viability and regeneration.

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REFERENCES