Research

Effect of Concentrated Growth Factor on Survival of Diced Cartilage Graft

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Abstract

Background: Diced cartilage grafts are important in rhinoplasty for raising the dorsum and eliminating dorsal irregularities. The most common problems with the use of diced cartilage are wrapping and cartilage resorption.

Objectives: To histopathologically investigate and compare the viability of diced cartilage grafts wrapped with concentrated growth factor, fascia and fenestrated fascia, or blood glue.

Methods: Cartilage grafts were harvested from the ears of 10 New Zealand White rabbits and diced into 0.5 to 1 mm^3 pieces. The grafts were divided into five groups for comparison: (1) bare diced cartilage; (2) diced cartilage wrapped with fascia; (3) diced cartilage wrapped with fenestrated fascia; (4) diced cartilage wrapped with concentrated growth factor (CGF); and (5) diced cartilage wrapped with blood glue. Each of the five grafts was autologously implanted into a subcutaneous pocket in the back of each rabbit. Three months later, the rabbits were sacrificed and the implants were harvested and examined histopathologically.

Results: Nucleus loss, calcification, inflammation, and giant cell formation differed significantly between the CGF group and both fascia groups. Chondrocyte proliferation was the highest in the CGF group. Nucleus loss rates were similar between the fascia and fenestrated fascia groups.

Conclusions: Our findings suggest that CGF improves the viability of diced cartilage grafts, while fascia hampers it. Punching holes in the fascia does not improve diced cartilage graft viability and neither does blood glue wrapping.

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Rhinoplasty is one of the most frequently performed cosmetic surgeries,[1] and autogenous cartilage grafts are commonly used in this surgery. Using diced cartilage grafts instead of blocks of cartilage grafts improves cartilage viability.[2] Additionally, diced cartilage grafts have the advantage of raising the dorsum and eliminating dorsal irregularities.

Although diced cartilage grafts were introduced by Peer as far back as 1941,[3] they were popularized in 2000 by Erol,[4] who used these grafts wrapped in Surgicel (Ethicon, Somerville, NJ), described as "Turkish delight," on the nasal dorsum in a broad series of patients. This unique method was associated with low revision surgery and graft resorption rates.[4] However, Daniel and Calvert later asserted that Surgicel reduced cartilage graft viability, and they proposed the use of deep temporal fascia for wrapping.[5] Research on this topic is ongoing, since complications such as scarring, alopecia, and hematoma were observed in the fascia donor area with both methods and because a new surgical field needs to be created for patient.

The most common problems in the use of diced cartilage grafts are the type of wrapping to use and cartilage resorption.[6] Studies conducted thus far have strived to increase the viability of cartilage grafts and achieve permanent and invisible cartilage grafts that can provide optimal coverage in the long term. Many clinical and experimental studies have been performed to test the use of Alloderm (LifeCell Corp, NJ), esterified hyaluronic acid, tensor fascia lata,

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autologous fibrin glue, rectus muscle fascia, Tisseel fibrin glue (Baxter, UK, and Newbury, UK), blood glue, and platelet-rich fibrin with these aims in mind.[7-14]

The five major growth factors (GFs) present in platelets and plasma are platelet-derived GF, fibroblast GF, transforming GF beta, vascular endothelial GF, and insulin-like GF, and platelet concentrates containing these factors are known to enhance tissue regeneration and healing.[15] The first generation of platelet concentrate, that is, platelet-rich plasma (PRP), is often used in clinical practice. Dohan et al defined the platelet-rich fibrin (PRF) matrix as the second generation of platelet concentrate.[16] Concentrated GF (CGF), defined by Sacco in 2006, is obtained by centrifugation of venous blood and it takes the form of a gel. PRF is similarly obtained from centrifugation of venous blood at varied speeds in a special centrifuge; it is denser than PRP and richer in GFs.[17,18]

Although some studies in the literature do discuss the use of diced cartilage grafts wrapped with fascia, to our knowledge, no study examines those wrapped with fenestrated fascia, which allows transition of plasma into the graft material from the recipient area. Oreroglu et al used gelatinous graft material obtained using diced cartilage grafts and bone dust with blood glue on the nasal dorsum.[13] However, no experimental studies examine the effects of blood glue on cartilage graft viability.

The aim of the present study is to histopathologically investigate the viability of diced cartilage grafts with various wraps: CGF, abundant in GFs and in a dense gel form; fascia and fenestrated fascia; and blood glue.

METHODS

Ten 6-month-old New Zealand White male rabbits weighing 2.670 to 3.100 g (mean: 2.900 g) were used as the experimental model. All surgical procedures were managed in accordance with the ethical principles and guidelines for experiments on animals approved by Pamukkale University Medical School Committee on the Use of Animal Subjects in Research. This experimental study was conducted between February 2015 and May 2015. All animal care and surgical procedures were performed humanely. The animals were housed in 12-hour light–dark cycles and provided feed pellets and water ad libitum.

Study Design

The study design is shown in Table 1.

Surgical Procedure

Each rabbit was anesthetized with an intramuscular injection of 35 mg/kg ketamine and 5 mg/kg xylazine hydrochloride. The right ears, backs, and thighs of the animals were shaved and disinfected with povidone iodine. All surgical procedures were performed under aseptic conditions.

Table 1. Study Design

Group	Graft Type
1	Diced cartilage only, bare
2	Diced cartilage with fascia
3	Diced cartilage with fenestrated fascia
4	Diced cartilage with CGF
5	Diced cartilage with blood glue

CGF, concentrated growth factor.

The right auricle from each rabbit was amputated and cartilage grafts were harvested without perichondrium. The grafts were divided into five pieces of 1 × 1 cm each, and each piece was diced into 0.5 to 1 mm^3 pieces using a No. 11 blade and placed in saline.

Next, two lumbosacral fascia grafts of dimensions 2 × 2 cm were collected via a 3-cm horizontal incision on the lower back region of the rabbits. The skin was sutured using 5/0 Vicryl sutures.

Additionally, seven 10-cc venous blood samples were obtained from the rabbits and placed in a sterile 10-mL blood tube without anticoagulants. These tubes were then immediately centrifuged in a special centrifuge device (Medifuge; Silfradent Srl, Sofia, Italy) for 13 minutes (Figure 1A). This device used a special program: 2700 rpm for 2 minutes, 2400 rpm for 4 minutes, 2700 rpm for 4 minutes, and 3000 rpm for 3 minutes. At the end of centrifugation, there were four fractions in the tube: (1) an uppermost serum layer (blood plasma without fibrinogen and coagulation factors); (2) a fibrin buffy coat (large and dense polymerized fibrin block); (3) a liquid layer containing GFs, white blood cells, and stem cells; and (4) a lowermost red blood cell layer. The fibrin buffy coat and liquid phase (second and third layers, respectively) were used in the experiments in this study. The red blood cell layer was separated from the fibrin block by using scissors (Figure 1B,C).

Graft Preparation

Five groups were formed depending on the type of graft used. Group 1 received bare diced cartilage grafts alone. Group 2 received iced cartilage grafts placed in a truncated tuberculin syringe. Fascia was wrapped around the syringe and the edges were closed using 6/0 Vicryl sutures. The grafts were then injected into the fascia and the ends was sutured with the same material (Figure 2A). Group 3 received cartilage grafts wrapped with fascia like group 2, but for this group, holes were created in the fascia using a No. 11 scalpel (Figure 2B). Group 4 received cartilage grafts covered with CGF (Figure 1D). Lastly, group 5 received

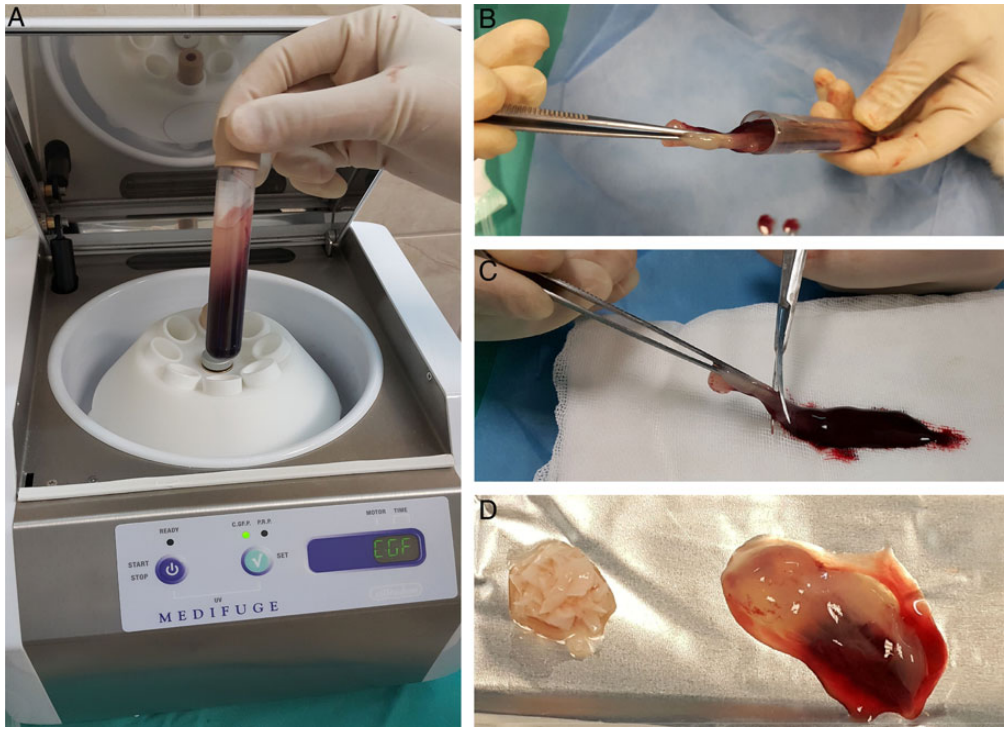

Figure 1. Preparation of CGF. (A) Venous blood samples without anticoagulants centrifuged in a special centrifuge device. (B,C) The fibrin block was separated from the red blood cell layer with scissors. (D) The appearance of diced cartilage and CGF.

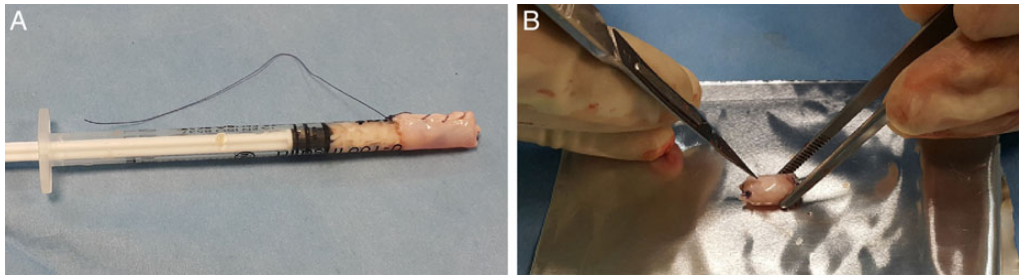

Figure 2. (A) Preparation of fascia wrapped diced cartilage graft. (B) Many holes were opened on the fascia wrapped diced cartilage graft by using a No. 11 scalpel.

cartilage grafts into which 1 cc blood was added dropwise. After 6 minutes, the blood coagulated and a gelatinous graft was obtained.

Figure 3 shows example grafts from each group.

Implant and Explant Procedures

Five skin incisions, approximately 1.5 cm long, were made in the paraspinal region of the rabbits, and small subcutaneous pockets were created. The five grafts were inserted into each of these pockets in the same rabbit, and the incisions were closed using 5/0 Vicryl sutures. No complications were seen at the recipient areas in the postoperative period. Three months after implantation, the rabbits were sacrificed with a high dose (150 mg/kg) of thiopental sodium. The implants were harvested and freed from the surrounding tissue.

Histopathologic Examination

Implant specimens were individually fixed in 10% formalin solution for 24 hours, dehydrated in ethyl alcohol solution, and cleared using xylol. They were then embedded in paraffin blocks, cut to 5-mm thickness, and stained with various stains. Hematoxylin-eosin (HE) staining was used to assess chondrocyte viability and the status of the chondroid tissue, while Masson's trichrome was used to demonstrate the collagen content of the matrix (collagen fibrils were stained green). Toluidine blue was used to assess metachromasia in

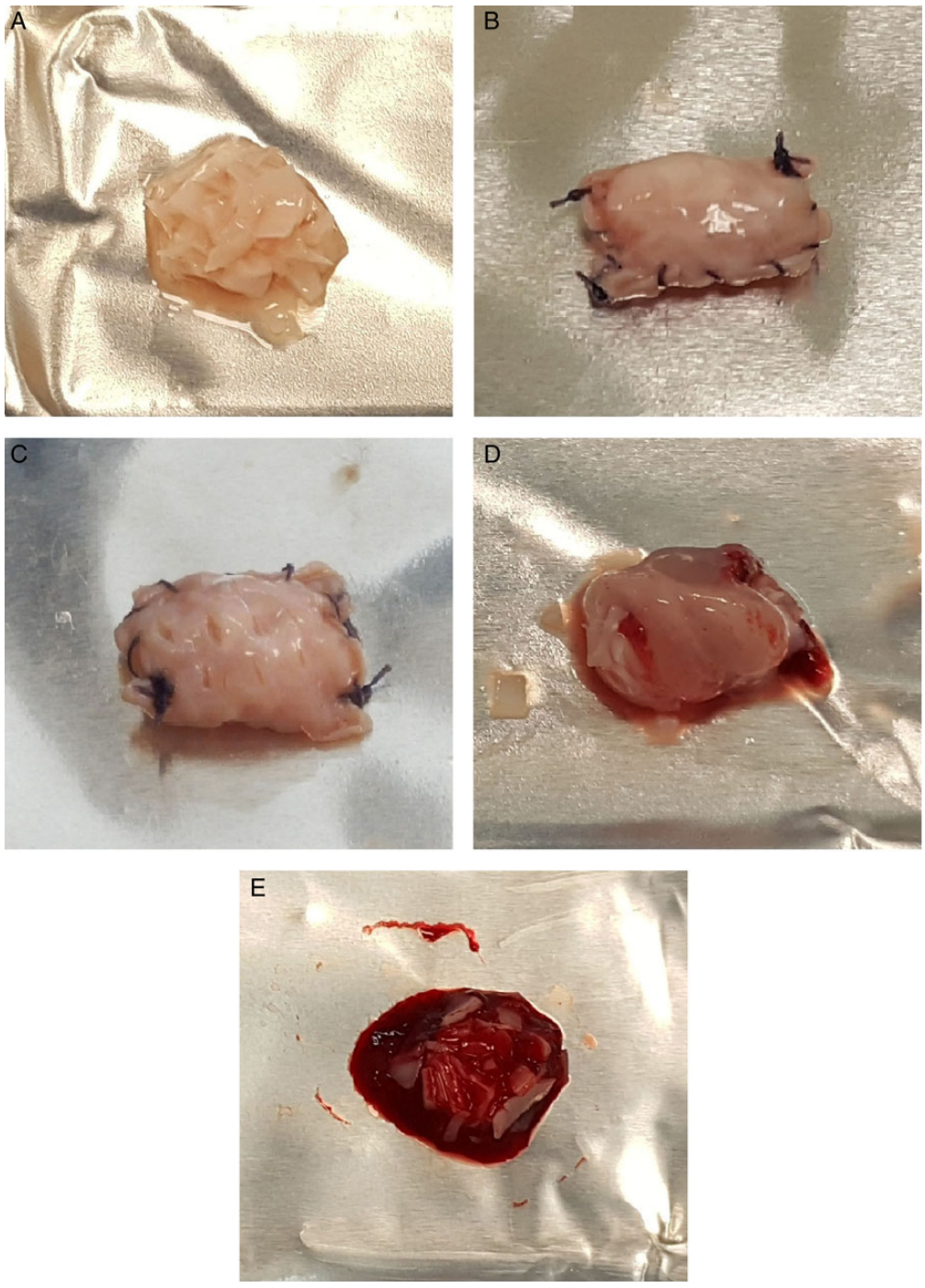

Figure 3. The five graft types. (A) Bare diced cartilage, (B) fascia wrapped diced cartilage, (C) fenestrated fascia wrapped diced cartilage, (D) graft wrapped with CGF, (E) graft wrapped with blood glue.

the chondroid tissue matrix and chondrocyte viability. Safranin-O staining reflected the proteoglycan content of the matrix: the matrix stains red, making it starkly distinguishable from the proteoglycan content, which stains green. Lastly, immunohistochemical staining of glial fibrillary acidic protein (GFAP) was used to demonstrate the regeneration potential of the chondrocytes. Brown deposition in the cytoplasm indicates positive staining for intermediate filaments.

Cartilage graft viability was determined on the basis of loss of matrix metachromasia and the absence of chondrocytic nuclei in the lacunae. The viability of each specimen was expressed as a percentage of the total tissue present.

Chondrocyte proliferation and transformation to connective tissue were assessed semiquantitatively. Neutrophil infiltration, cartilage mass loss, and chondrocyte loss were used as markers of cartilage resorption.

The specimens were examined under a light microscope using a 100× objective lens for HE-stained samples and the 200× objective lens for all other specimens. The pathologist performing the histological analysis was blind to the examination groups. Histopathological parameters, including loss of chondrocyte nuclei, peripheral proliferation, fibrosis, inflammation, degree of graft resorption, basophilia, giant cell formation, fragmentation, vascularization, tissue calcification, and bone metaplasia, were reviewed for all groups. Parameters were recorded as a percentage of all analyzed material: 0% (none) as 0, 1% to 25% (minimal) as 1+, 26% to 50% (moderate) as 2+, 51% to 75% (moderate–severe) as 3+ and 76% to 100% (severe) as 4+.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 17.0; SPSS, Chicago, IL). The Kruskal-Wallis test was used to investigate differences in histological findings among the groups. The Mann-Whitney U test was used for examine differences in the findings between groups. The results were considered significant at $P < 0.05$.

RESULTS

Table 2 shows the scores of the histopathological parameters in all, while Table 3 shows the comparison of these

Table 2. Histopathologic Parameters for Each Group

Histopathologic Parameters	Bare Diced Cartilage					With Fascia					With Fenestrated Fascia				
	n = 10					*n* = 10					*n* = 10				
	0	1+	2+	3+	4+	0	1+	2+	3+	4+	0	1+	2+	3+	4+
Loss of chondrocyte nucleus	–	–	1	8	1	–	–	1	4	5	–	–	1	6	3
Peripheral proliferation	–	1	7	–	2	1	2	5	1	1	–	2	5	2	1
Graft resorption	–	9	1	–	–	–	5	3	2	–	–	3	5	2	–
Calcification	6	4	–	–	–	2	4	2	1	1	2	5	3	–	–
Basophilia	–	4	4	2	–	–	5	3	2	–	–	5	4	1	–
Bone metaplasia	7	1	1	1	–	5	1	2	2	–	3	3	2	2	–
Inflammation	–	9	1	–	–	–	4	4	1	1	–	5	2	2	1
Fibrosis	–	6	4	–	–	–	2	7	1	–	–	3	5	2	–
Vascularization	–	7	3	–	–	–	4	4	2	–	–	4	2	4	–
Giant cell formation	9	1	–	–	–	1	1	2	5	1	–	3	3	2	2
Fragmentation	–	9	1	–	–	–	6	3	1	–	–	4	3	3	–
Histopathologic Parameters	**With CGF**					**With Blood Glue**									
	n = 10					*n* = 10									
	0	1+	2+	3+	4+	0	1+	2+	3+	4+					
Loss of chondrocyte nucleus	–	–	5	5	–	–	–	3	4	3					
Peripheral proliferation	–	–	3	6	1	–	2	4	3	1					
Graft resorption	–	8	2	–	–	–	7	2	1	–					
Calcification	6	4	–	–	–	4	5	–	1	–					
Basophilia	–	4	3	3	–	–	2	5	2	1					
Bone metaplasia	5	2	2	1	–	4	3	–	3	–					
Inflammation	–	10	–	–	–	–	8	2	–	–					
Fibrosis	–	5	4	1	–	–	2	7	1	–					
Vascularization	–	5	5	–	–	–	3	5	2	–					
Giant cell formation	6	3	–	–	1	4	4	1	1	–					
Fragmentation	–	8	2	–	–	–	7	3	–	–					

CGF, concentrated growth factor.

Table 3. Statistical Comparison of Histopathologic Parameters for Groups

Histopathologic Parameters	Statistical Comparison for Groups							
	Groups 1 and 2	Groups 1 and 3	Groups 1 and 4	Groups 1 and 5	Groups 2 and 3	Groups 2 and 4	Groups 2 and 5	Groups 4 and 5
Loss of chondrocyte nucleus	0.118	0.399	**0.042***	1.000	0.450	**0.008***	0.255	0.150
Peripheral proliferation	0.416	0.898	0.092	0.868	0.515	**0.031***	0.379	0.180
Graft resorption	**0.049***	**0.007***	0.542	0.255	0.516	0.127	0.365	0.549
Calcification	**0.023***	**0.032***	1000	0.306	0.574	**0.023***	0.159	0.306
Basophilia	0.744	0.565	0.809	0.332	0.836	0.598	0.214	0.497
Bone metaplasia	0.346	0.122	0.441	0.210	0.635	0.776	0.779	0.602
Inflammation	**0.020***	**0.044***	0.317	0.542	0.904	**0.005***	0.056	0.146
Fibrosis	0.058	0.112	0.547	0.058	0.966	0.251	1.000	0.251
Vascularization	0.126	0.078	0.374	0.054	0.630	0.403	0.744	0.208
Giant cell formation	**0.000***	**0.000***	0.121	**0.020***	0.725	**0.010***	**0.011***	0.408
Fragmentation	0.121	**0.018***	0.542	0.276	0.284	0.300	0.557	0.615

Group 1, bare diced cartilage; Group 2, fascia wrapped diced cartilage; Group 3, fenestrated fascia wrapped diced cartilage; Group 4, diced cartilage with CGF; Group 5, diced cartilage with blood glue.
*P value < 0.05: statistically significant.

parameters among the groups. Further, Figures 4-8 show the histopathological findings of all groups.

The loss of chondrocyte nuclei was the lowest in the CGF group, and this parameter differed significantly between the CGF and bare cartilage groups. Further, chondrocyte nucleus loss, calcification, inflammation, and giant cell formation were significantly lower in the CGF group than both fascia groups. The nucleus loss rates were similar in the fascia and fenestrated fascia groups, and no statistically significant differences were found in any parameter between the fascia and fenestrated fascia groups.

Chondrocyte proliferation was the highest in the CGF group and was moderate–severe. The bare cartilage graft group and both fascia groups showed similar levels of chondrocyte proliferation, which was moderate. Cartilage resorption and calcification were considerable in the fascia and fenestrated fascia groups and were minimal in the other three groups. Cartilage resorption, calcification, inflammation, and giant cell formation differed significantly between both fascia groups and the bare cartilage group. The extent of basophilia was similar among the groups and was minimal–moderate. Bone metaplasia, however, was the lowest in the bare cartilage group and was none–minimal in the other four groups.

Maximum inflammation was observed in the fascia and fenestrated fascia groups, while minimum inflammation was observed in the CGF, blood glue, and bare cartilage groups. Giant cell formation was at none–minimal in the CGF and blood glue groups, and none was observed in the bare cartilage group. However, it was severe in the fascia and fenestrated fascia groups. While fibrosis was moderate in the fascia and blood glue group, it was minimal–moderate in the other three groups. Minimum vascularization was observed in the bare cartilage group, and it was minimal–moderate in the fascia, CGF, and blood glue groups. The highest vascularization rate was seen in the fenestrated fascia group. Maximum fragmentation was observed in the fenestrated fascia group, and it was minimal in the CGF, blood glue, and bare cartilage groups.

DISCUSSION

One of the most important goals of rhinoplasty is acquiring a smooth and symmetrical nasal dorsum. Alloplastic materials can be used to augment the nasal dorsum or eliminate the dorsal irregularities, but the use of autologous materials is more common because of problems associated with alloplastic materials, such as the risk of infection, biocompatibility, and reconfiguration.[5]

Cartilage and other soft tissue such as temporal fascia, fat, and dermis are used in autologous grafts. Septal cartilage grafts are preferred, particularly in primary rhinoplasty,

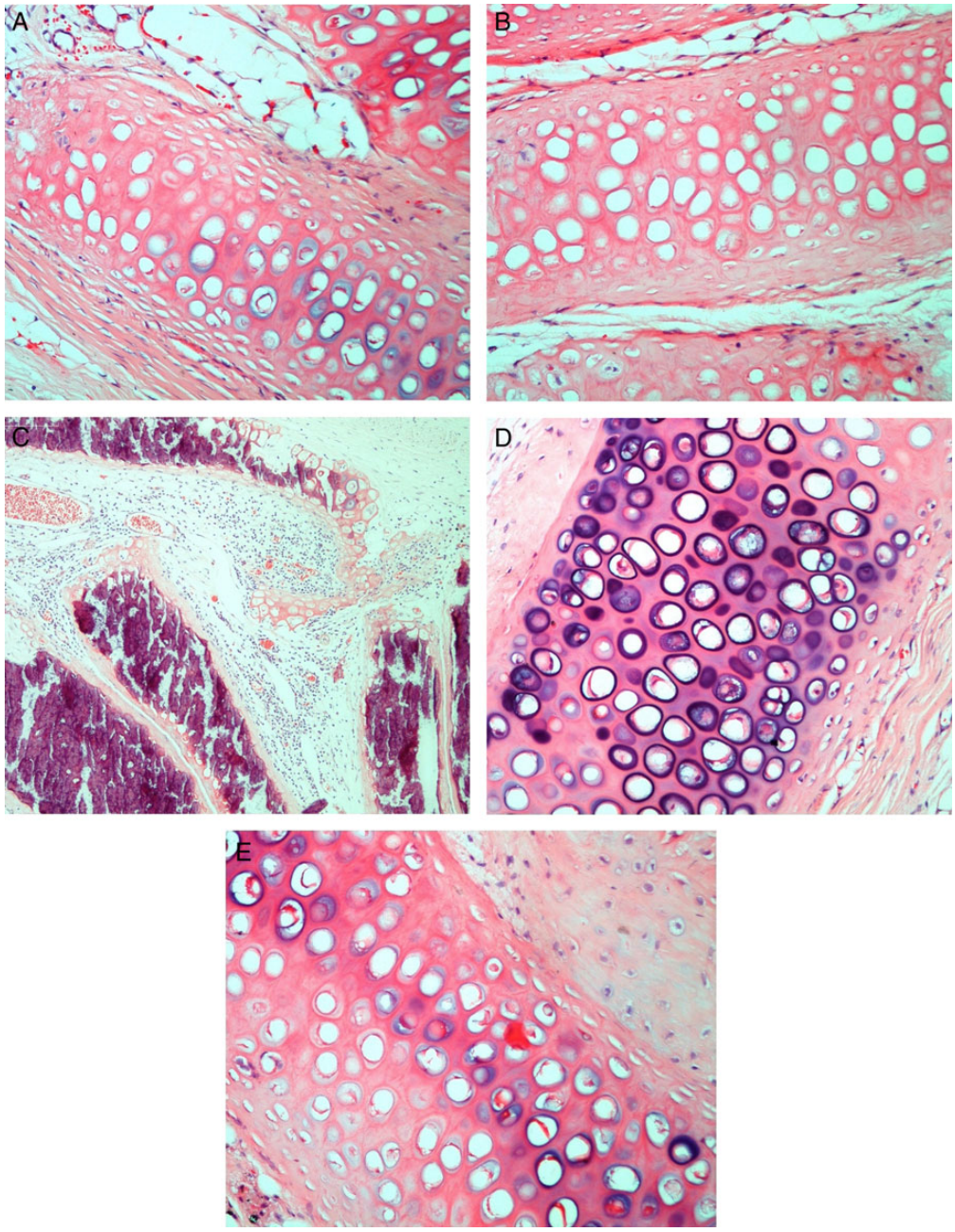

Figure 4. Hematoxylin-eosin stain of grafts; original magnification, × 100. This stain is the best marker of chondrocyte cell viability and status of chondroid tissue. (A) Bare diced cartilage graft. (B,C) Fascia wrapped and fenestrated fascia wrapped diced cartilage graft were showed similar histopathological results. Increased chondrocyte nucleus loss, calcification, inflammation, and giant cell formation were seen in these groups. (D) Graft wrapped with CGF. Nucleus loss of the chondrocytes was smallest in this group. Increased chondrocyte viability and peripheral proliferation were found in this group. (E) Graft wrapped with blood glue.

because they do not create additional donor site morbidity.[8] It is advantageous to use diced cartilage grafts in the nasal dorsum because block cartilage grafts are associated with problems such as resorption, distortion, and feeling by palpation, which are especially observed in the case of thin-skinned patients.[19]

Erol used diced cartilage grafts wrapped with Surgicel in the nasal dorsum of 2365 patients over a period of 10 years and reported successful long-term results for this technique.[4] However, subsequent clinical and experimental studies showed that Surgicel caused foreign body reactions and had negative effects on cartilage viability and resorption.[5,20,21]

Daniel and Calvert used diced cartilage grafts wrapped with temporal fascia in the nasal dorsum by modifying Erol's technique and reported more permanent clinical and histological long-term results than those found in Erol's

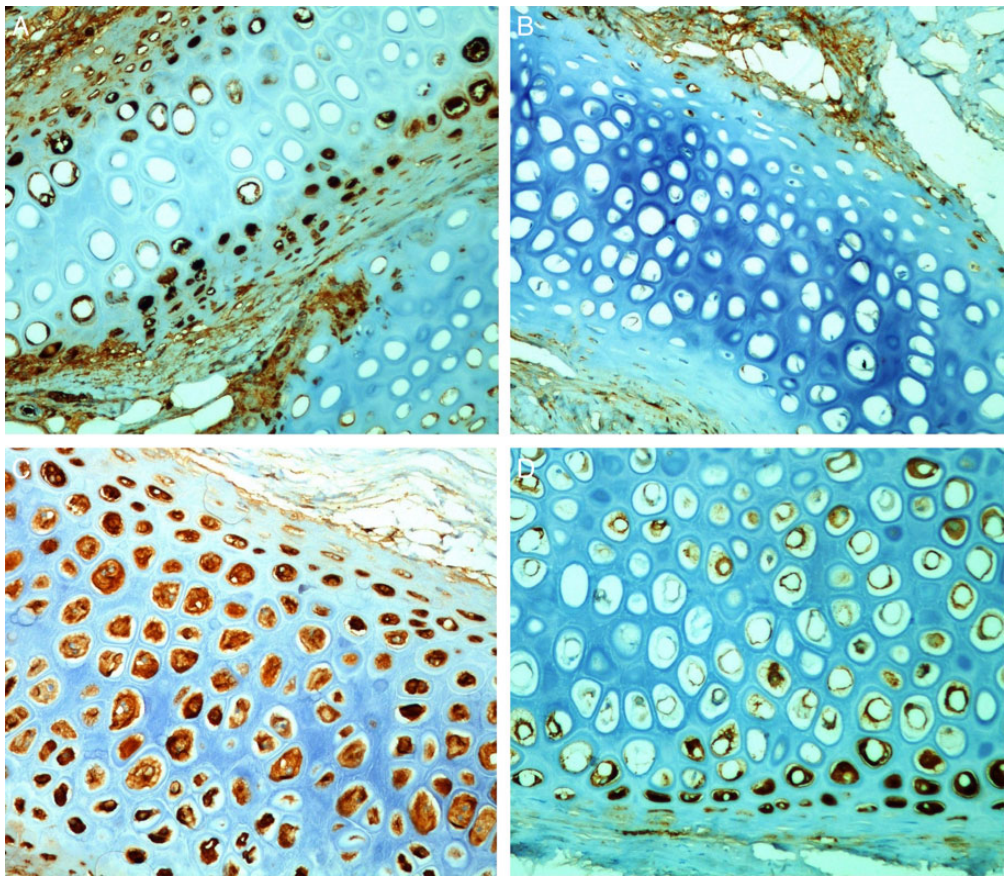

Figure 5. Glial fibrillary acidic protein immunohistochemical stain of graft; original magnification, × 200. Brown deposition in the cytoplasm indicates a positive staining for intermediate filaments. (A) Bare diced cartilage graft. (B) Fascia wrapped diced cartilage. (C) Graft wrapped with CGF. This group showed diffuse and strong positive reaction of chondrocyte (increased intracytoplasmic brown deposition). This demonstrates the regeneration potential of chondrocytes. (D) Graft wrapped with blood glue.

study.[5] However, even this improvised technique demonstrates problems such as hematoma, cicatricial alopecia, a prolonged operative time, and possible additional surgical site morbidity in the temporal fascia donor area, so studies continue to attempt to identify the optimal diced cartilage graft covering material and improve graft viability.[6]

Using an animal model of athymic nude Rowlett rats, Brenner et al found that the viability of diced cartilage grafts increased when they were wrapped with fascia. They reported that fascia increases chondrocyte survival, acting like perichondrium[6] but without the vascular structure that perichondrium has. It is known that cartilage grafts feed by plasmatic imbibition from the implanted area. When cartilage grafts are diced, their surface area increases as does the diffusion rate, because chondrocytes come into contact with the surrounding tissue. Fascia and other covering materials create a barrier to plasma transition between cartilage grafts and the recipient area, because of which chondrocyte viability is affected.[9] Some experimental studies show that bare cartilage grafts undergo less resorption than grafts wrapped with fascia.[2,22]

In the present study, for the first time, bare cartilage grafts were compared to cartilage grafts wrapped with fascia and fenestrated fascia. In the latter group, after the cartilage grafts were covered with fascia, holes were punched into the fascia with a No. 11 scalpel in order to ensure plasma transition into the graft. According to the histopathological findings, all parameters were similar in the fascia and fenestrated fascia groups. Thus, the presence of holes in the fascia did not affect cartilage viability.

Comparison of both fascia groups with the control group showed that cartilage resorption, calcification, inflammation, and giant cell formation were significantly higher in both fascia groups than the control group. Further, chondrocyte nucleus loss was lower in the bare cartilage group than the fascia groups, although this difference was not significant. These findings indicate that diced cartilage grafts wrapped with fascia or fenestrated fascia are poorly nourished because of nutrient diffusion from the grafted area, which affects their viability.

In some clinical studies, autologous materials acting as a scaffold were used instead of wrapping the diced cartilage

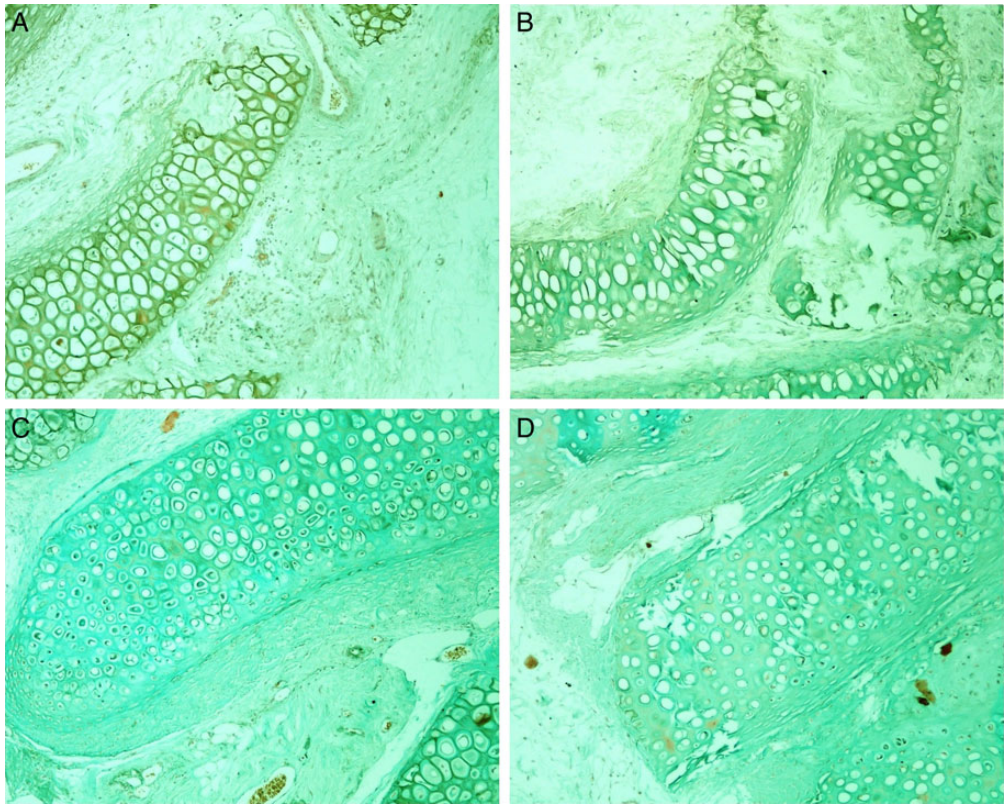

Figure 6. Masson's trichrome stain of grafts; original magnification, × 200. This stain demonstrate collagen content of the matrix by staining collagen fibrils green. (A) Bare diced cartilage graft. (B) Fascia wrapped diced cartilage. (C) Graft wrapped with CGF. (D) Graft wrapped with blood glue.

grafts. In this way, graft stabilization was ensured and good results were observed in the long term without prevention of graft diffusion. Bullocks et al used autologous tissue glue (ATG) derived from PRP and platelet-poor plasma during nasal dorsal augmentation and reported that the resultant gel structure was ideal because it provided GFs as well as graft stabilization.[10] However, bovine thrombin and calcium chloride had to be added for ATG activation, and the risk of anti-bovine thrombin antibody formation and the consequent risk of an immune response are the disadvantages of this technique.[23] Stevenson and Hodgkinson used Tisseel fibrin glue as a scaffold and reported long-term good results after secondary rhinoplasty because of the "cartilage putty," which was prepared by mixing morselized cartilage grafts with fibrin glue.[12] Tasman et al similarly used diced cartilage glue grafts for nasal dorsal augmentation by using fibrin glue.[24]

Platelet concentrates include GFs, which regulate the proliferation of cells via specific receptors. Vascular endothelial growth factor stimulates angiogenesis, platelet-derived growth factor is involved in mesenchymal cell survival and migration, tissue growth factor b triggers fibrosis, insulin-like growth factor retards cell apoptosis, and epidermal growth factor promotes cell proliferation and differentiation. PRP is the first-generation platelet concentrate and is obtained by adding anticoagulant, calcium chloride, and bovine thrombin to donor blood.[16] Because it is not in the form of a gel, it cannot serve as a scaffold for cartilage grafts.

PRF is the second-generation platelet concentrate.[16] Güler et al reported that an autologous PRF matrix increased cartilage viability in their rabbit model.[14] PRF is obtained by centrifugation of venous blood and it assumes a fibrin clot structure, rich in GFs. Goral et al investigated the effects of PRF on the viability of diced cartilage and found that PRF increases cartilage viability and serves as a graft carrier because of its gel form.[25]

CGF is obtained from fresh venous blood in the same manner as PRF but the blood is centrifuged at different speeds in a special centrifuge. It has a three-dimensional fibrin structure that is more intense and has better adhesion and regenerative capacity than PRF, and has a higher concentration of GFs.[17] Clinical and experimental studies show that CGF increases new bone formation when used with bone grafts in the healing of bone defects and is useful for sinus augmentation in dental surgery.[17,26] Honda et al investigated the effects of bone marrow stromal cells and

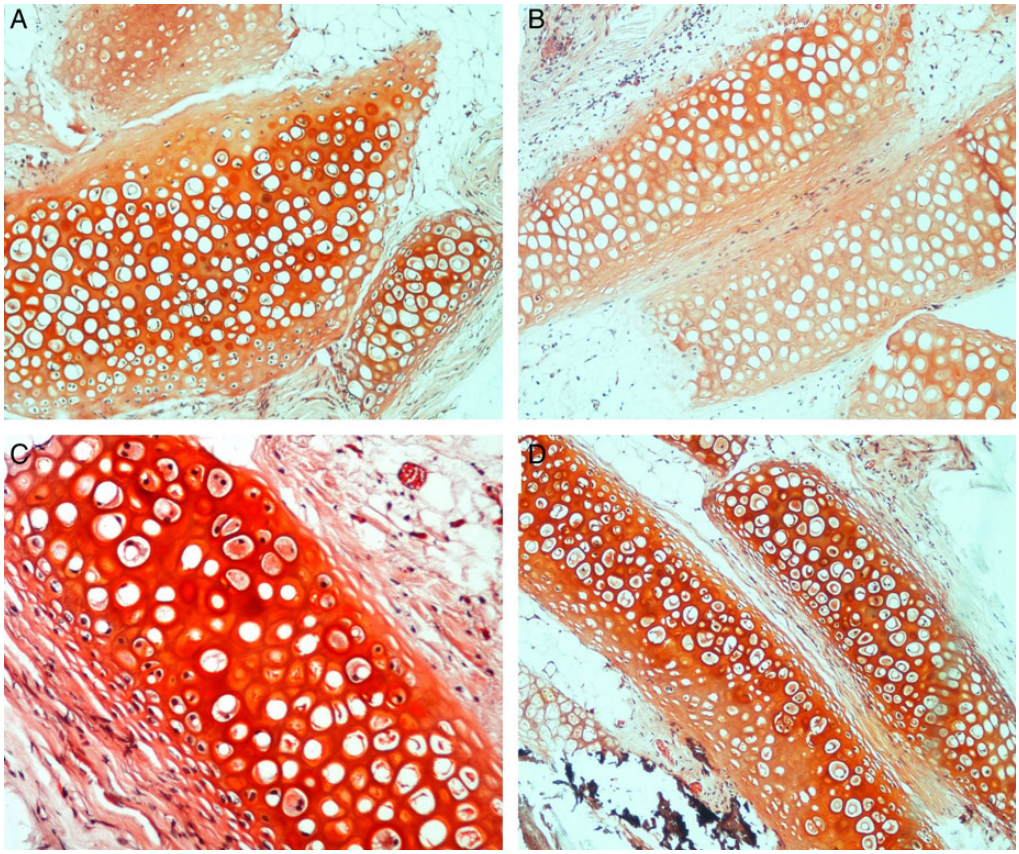

Figure 7. Safranin-O stain of grafts; original magnification, ×200. The red color in the matrix reveals the proteoglycan content of the matrix. (A) Bare diced cartilage graft. (B) Fascia wrapped diced cartilage. (C) Graft wrapped with CGF. (D) Graft wrapped with blood glue.

CGF in an experimental model of bone defects and reported that CGF provided a good scaffold to facilitate bone regeneration. The authors emphasize the three advantages of CGF: (1) CGF includes cytokines, which stimulate cell maturation and matrix production; (2) preparation of CGF and migration of cells into CGF are quick and easy; and (3) it is reliable because it does not have any other components except autologous blood.[27] Bujia et al also found that GFs increased the proliferation of chondrocytes in vitro.[28]

In the present study, we investigated the effects of CGF on the viability of diced cartilage grafts, which have not been discussed previously in the literature. Many experimental studies have histologically examined diced cartilage viability,[2,6,8,9,14,20-22,25] and this viability is considered to reflect long-term survival. In the present study, histopathological examination showed that nucleus loss in the CGF group was significantly lower than that in the bare diced cartilage group. Peripheral cartilage proliferation was also higher in the CGF group. Thus, CGF seems to enhance diced cartilage graft viability. Compared to CGF, diced cartilage wrapped in fascia, which finds common clinical use, showed significantly lower cartilage viability. Although CGF does not provide as solid a wrap as fascia, it seems an ideal replacement. Further, the extent of inflammation, giant cell formation, and calcification was considerable in the fascia group. Besides these advantages, CGF is easier to obtain than fascia, does not create donor site morbidity, and does not extend the operating time. The only disadvantage is that its preparation requires a special centrifuge.

The ideal wrapping material for diced cartilage grafts remains to be identified. CGF is a good solution to two cartilage graft-related problems, that is, wrapping and resorption, and it may therefore be suitable as an autologous material for graft carriage. During rhinoplasty operations a greater amount of blood can be received and a sufficient amount CGF can be obtained from the patient. Thus, adequate wrapped diced cartilage graft material can be obtained for use in the nasal dorsum. Additionally, CGF has the advantage of improving cartilage viability and therefore long-term graft survival. This solves for dorsal irregularities, which are one of the long-term complications of rhinoplasty. In the future, we plan to conduct a clinical study on the use of CGF in rhinoplasty.

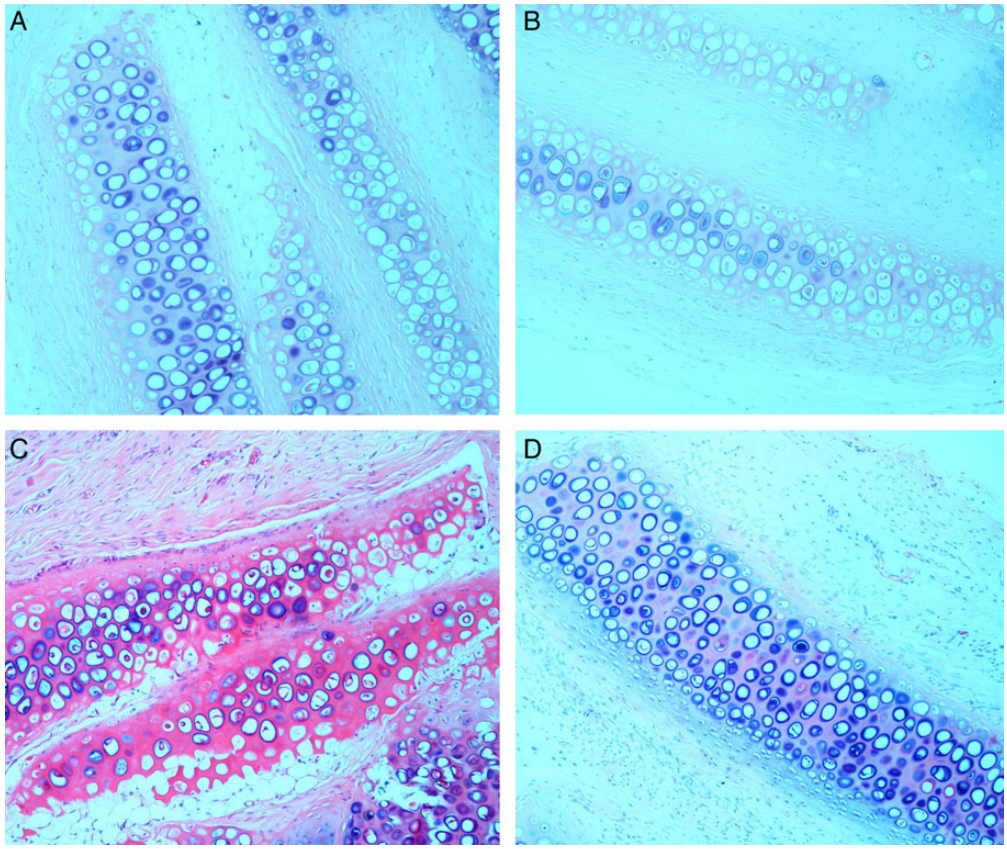

Figure 8. Toluidine blue stain of grafts; original magnification, × 200. This stain demonstrate chondroid tissue matrix metachromasis and chondrocyte viability by staining matrix blue. (A) Bare diced cartilage graft. (B) Fascia wrapped diced cartilage. (C) Graft wrapped with CGF. (D) Graft wrapped with blood glue.

Oreroglu et al applied venous blood from a peripheral vein dropwise into a mixture of diced cartilage graft and bone dust and used this easily shaped gelatinous graft mixture for the nasal dorsum.[13] However, they did not conduct histopathological examination in their study. In the present study, we investigated the effects of venous blood glue on diced cartilage grafts, and histopathological examination showed that only giant cell formation differed significantly between the blood glue group and other groups: it was greater in the bare cartilage group and lower in both fascia groups. Although the differences in nucleus loss and peripheral proliferation rate were not significant, the results were better in both fascia groups than in the blood glue group, and the CGF group showed neither of these outcomes. On the basis of our findings, we believe that blood glue does not significantly improve cartilage viability but can act as a scaffold.

Our study has some limitations. The amount of venous blood that could be harvested for CGF preparation was limited in the experimental rabbit model. To avoid morbidity related to blood loss, no more than 10 cc blood was collected from each rabbit. Better cartilage graft stabilization and better regeneration may be achieved with a greater amount of CGF.

CONCLUSIONS

Our histological study showed that compared to fascia, CGF increases the viability of diced cartilage grafts and has the advantages of being easy to prepare and apply and not causing donor site morbidity. However, these results must be supported by long-term clinical trials.

Although blood glue does not significantly improve diced cartilage graft viability, it is the easiest wrapping material to prepare and functions effectively as a scaffold. Wrapping of diced cartilage grafts with fascia affects the viability negatively by reducing nutrient diffusion, and punching holes in the fascia does not positively affect viability.

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