

The Effects of Mucoperichondrial Flap Elevation on Septal L-Strut Cartilage: A Biomechanical and Histologic Analysis in a Rabbit Model

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Background: The harvesting of septal cartilage following mucoperichondrial flap elevation has almost become a standard step in rhinoplasty. However, the strength of the remaining septum has not yet been evaluated. In the current experimental study of a rabbit rhinoplasty model, the remaining septum following a graft harvest was analyzed both biomechanically and pathologically.

Methods: Forty New Zealand rabbits were classified into four equal groups. Group 1 consisted of the animals in which unilateral elevation of the mucoperichondrial flaps was undertaken before the graft harvest, group 2 consisted of the animals in which bilateral elevation was undertaken, group 3 included the animals where the septum was exposed and left untouched after a bilateral mucosal flap elevation, and group 4 was designated as the control group. Specimens were analyzed under light microscopy for multiple parameters. Biomechanical analyses were performed with a universal testing device at the Department of Engineering, Biomechanical Laboratories, Istanbul Technical University.

Results: Biomechanical analysis in terms of maximum tension revealed significant results among the groups ($p = 0.008$). Although insignificant results were observed overall using a pathologic analysis, the amount of chondrocytes was lower in group 2 than in group 1 ($p = 0.099$). The amounts of matrix collagen ($p = 0.184$) and fibrosis were ($p = 0.749$) higher in group 2 than in group 1.

Conclusions: From these data, the authors conclude that mucoperichondrium integrity plays a crucial role in the biomechanical strength of the septum. More sophisticated studies with further pathologic analysis are required to determine the exact mechanism of strength loss observed with mucoperichondrial flap elevation. (*Plast. Reconstr. Surg.* 137: 1784, 2016.)

Currently, septoplasty is considered a crucial step of performing a rhinoplasty. The opening of the air passage and the restoration of the septum at the midline depend on a well-performed septal operation. In addition, many surgeons perform septal intervention to harvest graft material in rhinoplasty. Theoretically, as a longstanding rule, graft material can be harvested by leaving cartilage portions of at least 1 cm at the dorsal and caudal

areas.¹ However, there remain questions concerning the histologic and biomechanical quality of the remaining cartilage following this maneuver. Almost all of the published literature focuses on the functional or aesthetic results achieved after rhinoplasty.² Hundreds of different grafts, suturing techniques, and surgical procedure modifications have been described in these articles.³

However, data regarding the quality, strength, and histology of the remaining cartilage after septal graft harvest are lacking. There are very few objective histopathologic evaluations on this, and no biomechanical evaluation has been performed to date.⁴ In view of these limited data, we aimed to provide objective biomechanical and histologic analyses of the remaining septum after graft harvest in an experimental rabbit rhinoplasty model.

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MATERIALS AND METHODS

After approval from the ethics committee, 40 New Zealand rabbits were divided equally into four groups of 10 animals each (Fig. 1). The rabbits weighed 3500 to 4500 g and there was no discrimination based on sex. The four groups were as follows:

1. Group 1: Unilateral elevation of the mucoperichondrial flaps of the septum followed by graft harvest, leaving an L-strut in the rabbit rhinoplasty model ($n = 10$).
2. Group 2: Bilateral elevation of the mucoperichondrial flaps of the septum followed by graft harvest, leaving an L-strut in the same model ($n = 10$).
3. Group 3: Bilateral elevation of the mucoperichondrial flaps of the septum, leaving the cartilage untouched, with follow-up in vivo of 6 months ($n = 10$).

4. Group 4: No elevation of the mucoperichondrial flaps and the cartilage left untouched, the cartilage totally excised in a single session, with no in vivo follow-up ($n = 10$) (control group).

Surgical Procedure

Surgical anesthesia was administered with intramuscular xylazine (5 mg/kg), ketamine (35 mg/kg), and 2 ml of 2% lidocaine with 1:100,000 epinephrine (Figs. 2 and 3). The nasal skin of the rabbits was shaved, and the field was prepared in the standard surgical fashion. A longitudinal 3-cm incision was made over the dorsum of the nose. The bone periosteum was exposed. The periosteum was incised and dissection was continued bilaterally in the subperiosteal plane using a sharp dissector. A longitudinal osteotomy was made in the midline using a 4-mm chisel. At this stage, bilateral median osteotomies were

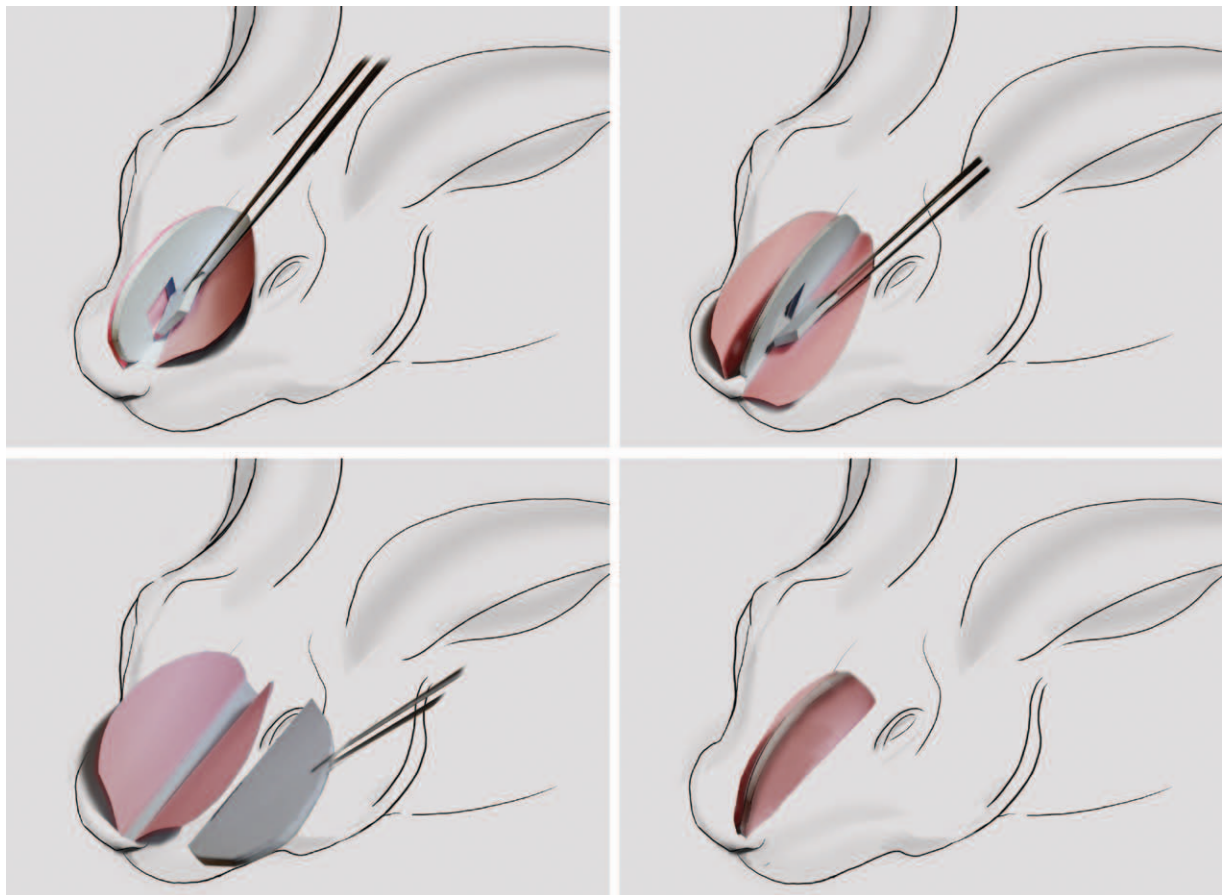


Fig. 1. Study design. (Above, left) Group 1: graft harvesting was performed by unilateral elevation of the mucoperichondrial flap. (Above, right) Group 2: harvesting was undertaken by bilateral mucoperichondrial elevation. (Below, left) Group 3: bilateral elevation of the mucoperichondrial flaps, leaving the cartilage intact, with follow-up in vivo of 6 months. (Below, right) Group 4: no elevation of the mucoperichondrial flaps and the cartilage was left intact, but the cartilage was totally excised without a follow-up in vivo.

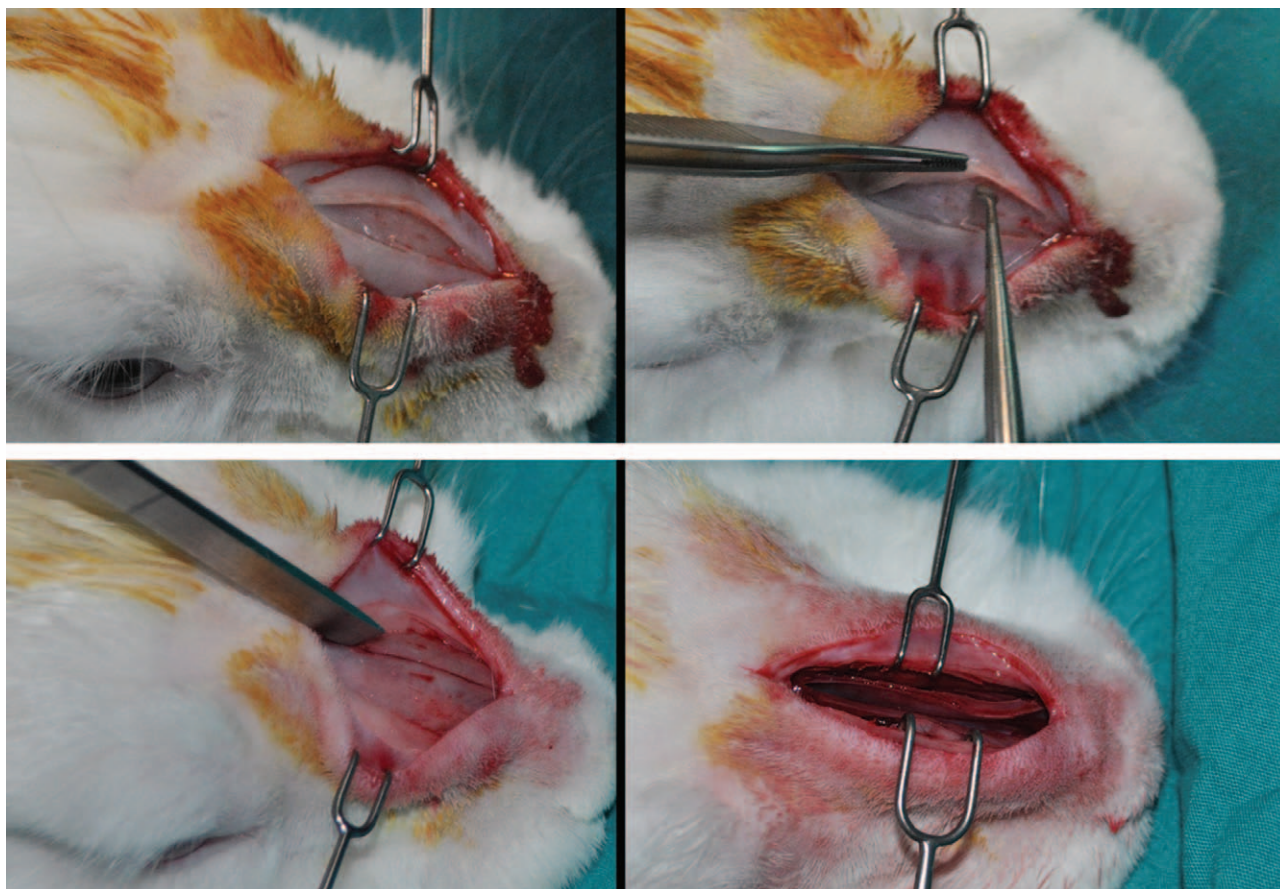


Fig. 2. (Above, left) A longitudinal incision was performed over the periosteum, exposing the nasal bone on the midline. (Above, right) Dissection proceeded on the subperiosteal plane using a sharp elevator. (Below, left) A longitudinal osteotomy was made on the midline with a 4-mm chisel. (Below, right) The septum was visualized following lateralization of the bone fragments with double hook retractors.

made to ensure maximum exposure. The septum was exposed in the midline by lateralizing the bone fragments.

An incision was made in the anterior perichondrium of the septum. The submucoperichondrial area was dissected using a Cottle elevator, and the mucoperichondrial flaps were elevated. This procedure was performed unilaterally in group 1 and bilaterally in group 2. After elevation of the flaps, 0.5 × 0.5-cm cartilage grafts were harvested, leaving an L-strut dorsally and caudally (groups 1 and 2). After irrigation and aspiration, the mucosal flaps were apposed together by transseptal suturing. The periosteum was approximated with a 4-0 polydioxanone suture. The skin was closed using absorbable 4-0 polyglactin sutures.

In group 3, the mucoperichondrial flaps were elevated bilaterally. Mucosal repair was performed again without harvesting the cartilage graft from the septum. The rabbits in groups 1, 2, and 3 underwent scheduled follow-up. In group 4, the

animals were killed after excision of the septal cartilage in a single session without any further procedures or follow-up (control group). All animals received postoperative analgesics with buprenorphine (0.04 mg/kg) and flunixin (1.1 mg/kg) along with postoperative sulfadoxine (200 mg) and trimethoprim (40 mg) intramuscularly two times.

After 6 months, the rabbits in both groups were anesthetized with intramuscular xylazine (5 mg/kg) and ketamine (35 mg/kg). An incision was made along the previous incision line and the mucoperichondrial flaps were reelevated. The L-strut was exposed, and the septal cartilage was excised in its entirety. All rabbits were killed using high doses of anesthetics.

Pathologic Analysis

The excised cartilages were subjected to standard fixation, dehydrated with graduated ethanol solutions, and embedded in paraffin; and the sections were stained with hematoxylin and eosin,

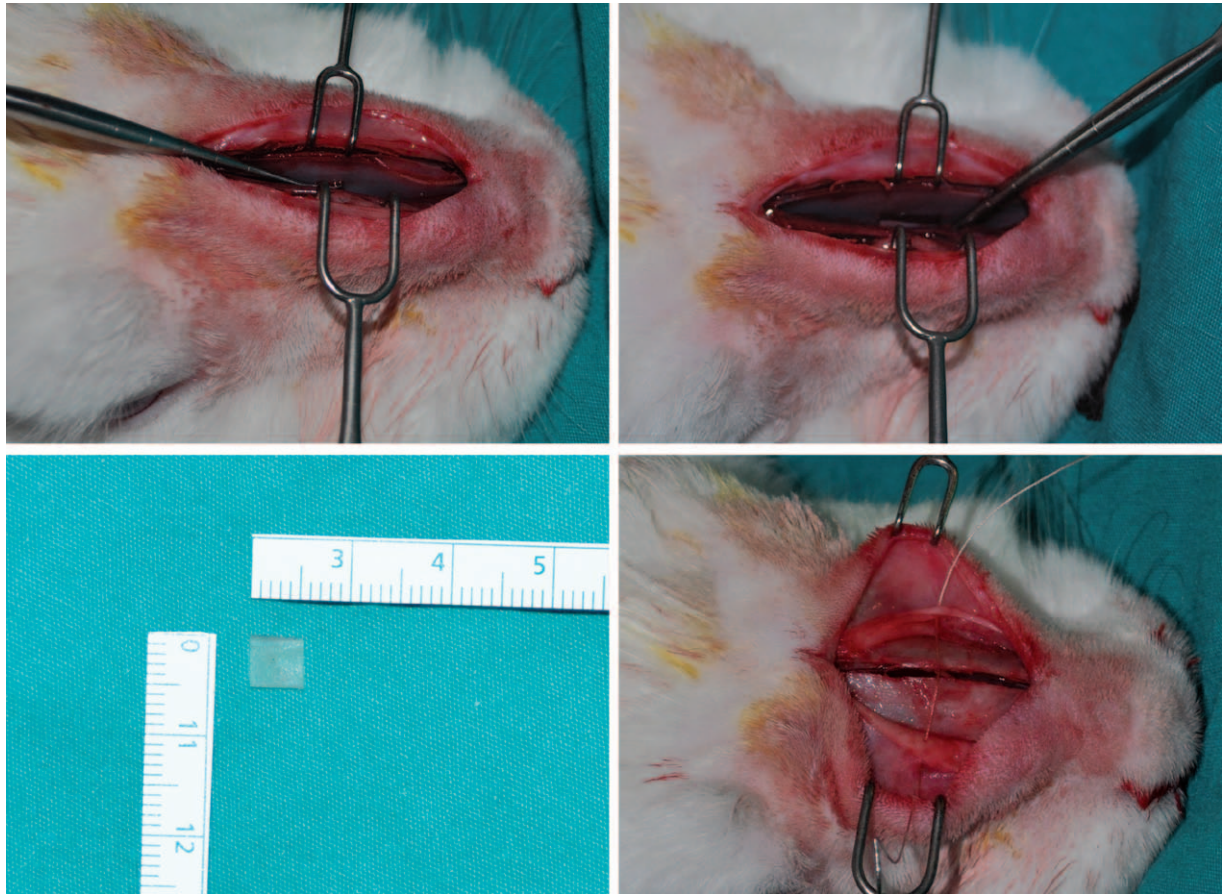


Fig. 3. (Above, left) Subperichondrial dissection was undertaken by a Cottle elevator. (Above, right) After elevation of the flaps (unilateral or bilateral), a cartilage graft was harvested by leaving an L-strut. (Below, left) The harvested 0.5 × 0.5-cm septal graft. (Below, right) The periosteum was approximated with 4-0 polydioxanone, and the skin was closed with 4-0 polyglactin sutures.

periodic acid–Schiff, toluidine blue, Masson trichrome, safranin-O, and Evans van Gieson stains followed by examination under light microscopy. Examination was performed for the parameters of chondrocyte count, inflammation, thickness, chondrocyte nuclei, peripheral proliferation, potential for regeneration, matrix collagen quantity, elastic fibril quantity, fibrosis, vascularization, fibrosis, and metaplastic bone development. This procedure was performed by a blinded pathologist. The obtained results were biostatistically compared.

Biomechanical Study

The septal cartilages were wrapped in gauze damped with isotonic saline, frozen under deep-freeze conditions, and sent to the biomechanical test and research laboratory, where they were slowly thawed at ambient temperature (Fig. 4). The samples were then carefully mounted in the universal test machine. A total of 40 samples were appropriately prepared for testing (Fig. 4).

Biomechanical experiments were conducted at the Biomechanics Laboratories of Istanbul Technical University using an MTS 858 Mini Bionix II (model no. 359.XX, part no. 100-146-714, Rev A, serial no. 10189576; MTS Systems, Inc., Eden Prairie, Minn.) universal test machine. Axial tension test software was developed based on the appropriate speed and force values by designing a loading scenario. Tension experiments were conducted using an ESIT Bending Beam Load Cell (serial 592, model SPA-10 kg, output 2.0 mV/V; Esit Electronic Ltd. Co., Istanbul, Turkey). During the tension experiment, the advancement speed of the universal test machine's moving jaw was set at 10 mm/minute, and the sampling speed was set at 10 Hz. Time, axial displacement, and axial force values were recorded as data during the tension test.

Experiments were continued until the samples were damaged. Load-displacement curves were plotted based on the data using MATLAB

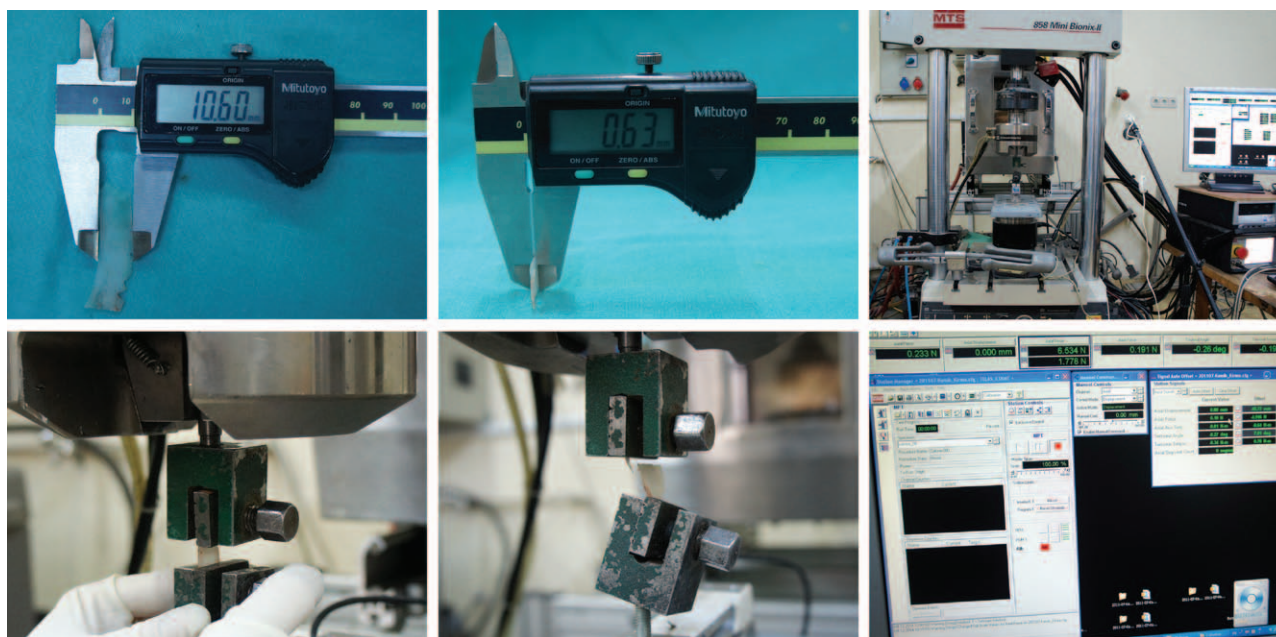


Fig. 4. (Above, left and center) The sample sizes were measured for each cartilage. The surface area measurements were entered into the system individually to obtain maximum tension values. (Above, right) Biomechanical experiments were conducted using an MTS 858 Mini Bionix II universal test machine, together with axial tension test software. Tension experiments were conducted using an ESIT Bending Beam Load Cell. (Below, left) The samples were secured carefully into place with the custom-made jaws. (Below, center) The advancement speed of the moving jaw of the universal test machine was set at 10 mm/minute and the sampling speed was set at 10 Hz. Experiments continued until the samples were damaged, as demonstrated. (Below, right) Axial tension test software was used based on the appropriate speed and force values.

software (The MathWorks, Inc., Natick, Mass.). These curves were used to calculate breaking loads and displacements at the breaking points. Section sizes were also measured for each sample. The tension values were found for each sample by calculating the section areas. Statistical evaluations were made according to these values, and numerical results were obtained.

Statistical Analysis

In this study, statistical analyses were performed using the NCSS 2007 Statistical Software (NCSS, Kaysville, Utah) software package. The data were evaluated using descriptive statistical methods (means and standard deviations) and the Kruskal-Wallis test for intergroup comparisons, Dunn's multiple comparison test for subgroup comparisons, and the chi-square test for qualitative data comparisons. The results were evaluated at a significance level of $p < 0.05$.

RESULTS

Histologic Results

In the histologic evaluation, the distribution differences in the parameters of chondrocyte

count, inflammation, thickness, chondrocyte nuclei, peripheral proliferation, potential for regeneration, matrix collagen quantity, elastic fibril quantity, fibrosis, vascularization, fibrosis, and metaplastic bone development were semi-quantitatively evaluated between the four groups, and a statistical evaluation was performed (Fig. 5).

In terms of the chondrocyte amount, no statistically significant results were found ($p = 0.099$), although the count was decreased in group 2 (mean, 1.1 ± 0.32) compared with group 1 (mean, 1.4 ± 0.52). The matrix collagen quantity was increased in group 2 (1.3 ± 0.48) compared with group 1 (1 ± 0 ; $p = 0.184$).

Although fibrosis was increased in group 2 (1.2 ± 0.42) compared with group 1 (1.1 ± 0.32), this was not statistically significant ($p = 0.749$). The other parameters that were not statistically significant were inflammation ($p = 0.360$), peripheral proliferation ($p = 0.368$), regeneration ($p = 0.126$), vascularization ($p = 0.362$), and metaplastic bone formation ($p = 0.374$).

Biomechanical Analysis Results

Statistically significant differences were found between the maximum force (in newtons) means of

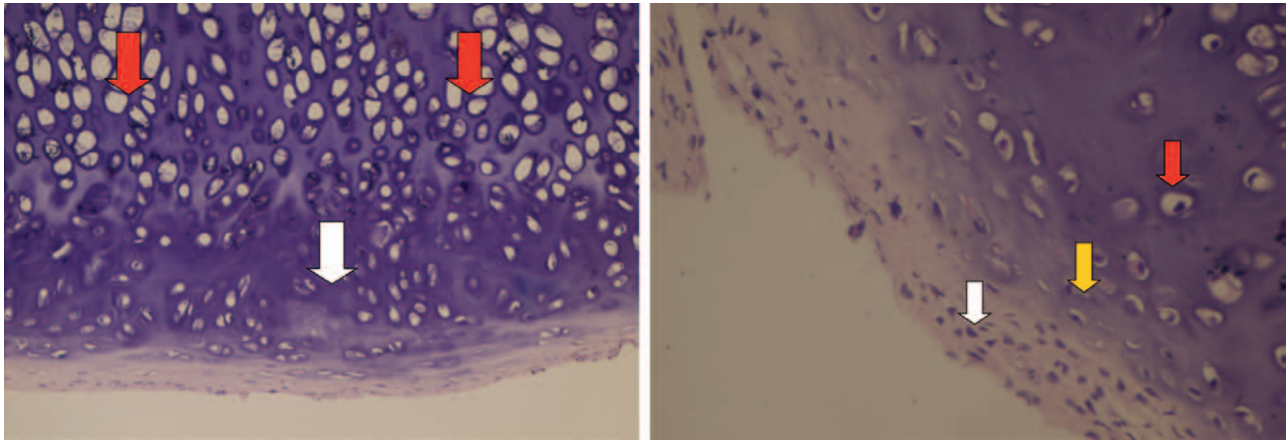


Fig. 5. (Left) Specimen showing peripheral cell proliferation with an increased number of nuclei (seen mostly in groups 1 through 3 as a response to injury). The *red arrows* indicate normal mature chondrocytes, whereas the *white arrow* demonstrates proliferating chondrocytes (hematoxylin and eosin; original magnification, $\times 10$). (Right) Inflammatory cells in the group 2 specimen. These cells can be considered a response to trauma, which was most obvious in group 2 (bilateral mucosa dissection group). The *red arrow* indicates chondrocytes, the *yellow arrow* indicates increased cellularity, and the *white arrow* demonstrates the inflammatory cells (lymphocytes, macrocytes, and small amount of neutrophils) (hematoxylin and eosin; original magnification, $\times 20$).

groups 1, 2, 3, and 4 ($p = 0.0001$) (Tables 1 and 2). In paired group analyses, the maximum force (in newtons) means of group 4 were statistically significantly higher than those of groups 1, 2, and 3 ($p = 0.013$, $p = 0.002$, and $p = 0.012$, respectively).

Statistically significant differences were found between the maximum tension means of groups 1, 2, 3, and 4 ($p = 0.008$). On paired group analyses, the maximum tension means of group 3 were statistically significantly higher than those of group 2 ($p = 0.005$).

Statistically significant differences were found between the displacement at maximum tension means of groups 1, 2, 3, and 4 ($p = 0.008$). On paired group analyses, the displacement at maximum tension means of group 2 were found to be statistically significantly lower than those of groups 3 and 4 ($p = 0.017$ and $p = 0.009$, respectively).

DISCUSSION

Septal cartilage grafts are frequently required in rhinoplasty, both for supporting the nasal structures and achieving aesthetic integrity. Although

septal cartilage is considered an ideal graft donor site, it should be noted that it is also the most important anatomical supporting structure in a nose.⁵

When the septal cartilage is examined histologically, it is seen that the mucoperichondrial area consists of four layers.⁶ The cells and extracellular matrix indirectly originate from the mesenchymal cells surrounding the perichondrium, forming the innermost layer, which is thought to be the most important layer in terms of a septal operation.⁷⁻⁹ Therefore, the subperichondrial plane is the most desirable plane to approach in terms of ensuring the integrity of this layer.¹⁰⁻¹² Moreover, dissection made in the subperichondrial plane ensures a bloodless surgical field and preserves the nasopalatine and incisive nerves and the specific structures in the vomeronasal and submucosal organs.¹⁰

In the literature, many studies have emphasized the importance of perichondrial integrity for the remaining cartilage left in vivo. For example, Verwoerd-Verhoef et al. demonstrated new cartilage formation within 2 weeks after submucosal cartilage resection in young rabbits.¹³ Duncan et al.¹⁴ demonstrated that the harvesting of

Table 1. Maximum Force, Maximum Tension, and the Displacement at Maximum Tension Values Obtained on Biomechanical Analysis*

	Group 1	Group 2	Group 3	Group 4	<i>p</i>
No.	10	10	10	10	
Maximum force, N	8.35 ± 3.05	6.06 ± 2.84	7.49 ± 2.23	14.71 ± 5.51	0.0001
Maximum tension, MPa	1.86 ± 0.82	1.27 ± 0.4	2.48 ± 0.76	2.21 ± 1.09	0.008
Displacement at maximum tension	2.74 ± 1.02	2.34 ± 0.53	3.45 ± 1.09	3.52 ± 1.79	0.032

*Maximum tension values were taken into account in this study because of different surface areas of specimens.

Table 2. Intergroup Analysis of Biomechanical Testing Results

Dunn's Multiple Comparison Test	Maximum Force (N)	Maximum Tension (MPa)	Displacement at Maximum Tension
Groups 1 and 2	0.065	0.097	0.460
Groups 1 and 3	0.620	0.069	0.137
Groups 1 and 4	0.013	0.680	0.215
Groups 2 and 3	0.143	0.005	0.009
Groups 2 and 4	0.002	0.054	0.017
Groups 3 and 4	0.012	0.674	0.674

cartilage grafts by preserving the perichondrium positively affected both graft viability and intercartilaginous recovery. Additional studies have demonstrated the corrective effects of the perichondrium at the head and neck region on the cartilage structures.¹⁵⁻¹⁷

Although it has been histologically demonstrated that preserving the mucoperichondrium positively affects the cartilage,^{14,18} no biomechanical evaluation has been performed on the effect of unilateral or bilateral elevation of mucoperichondrial flaps on the strength of the remaining septal cartilage. This can be considered the starting point of our study.

In our study, no statistically significant differences were found between the groups in terms of chondrocyte count, inflammation, peripheral proliferation, matrix collagen quantity, vascularization, fibrosis, and metaplastic bone development. When the chondrocyte count was examined alone, reduced chondrocyte counts were found in group 2 compared with group 1. This may be explained by the increased damage to chondrocytes because of an additional mucoperichondrial dissection. However, the statistical insignificance of this reduction may be explained by the low turnover rate of the chondrocytes located in the central zone of the cartilage.¹⁹ It may be hypothesized that the limited 6-month period of the study, just like the findings in similar studies, may fail to show the damage caused by mucoperichondrial flap elevation on the septal cartilage.

When group 2 was compared with group 1, an increase in the matrix collagen quantities was noted. This increase is a response to the trauma and may be meaningful in terms of causing a harder and nonelastic septum cartilage. This is, in fact, an expected response. However, no statistically significant results could be obtained. The increase in another parameter (i.e., fibrosis) was also in parallel with the above considerations. The increase in fibrosis, albeit statistically insignificant, in group 2, in which more mucoperichondrial dissection was

performed, may be meaningful in demonstrating the damage caused by the trauma on the cartilage.

When it is assumed, in terms of biomechanical analysis, that the sample sections were standardized, the maximal force should be used as the evaluation parameter. However, in our study, maximal tension was used as the basis because the remaining cartilages after graft harvesting were of different lengths and thicknesses. Biomechanically, these findings showed that the detachment of the septum mucoperichondrial flaps from the cartilage progressively weakened the septum ($p = 0.008$). The significant reduction in the cartilage tension should also be investigated by intergroup analysis in addition to general evaluation. The aim would be to form an opinion about whether the loss of tension is caused by mucoperichondrial dissection or by harvesting or damaging the cartilage graft. That is to say, in the comparison between groups 1 and 2, which rather demonstrates the effect of mucosal dissection, a reduction was observed in group 2, albeit insignificant ($p = 0.097$). Moreover, in the group 2 and group 4 analysis, which is meaningful in terms of demonstrating the additive effect (mucoperichondrial dissection and graft harvesting), serious reductions were observed ($p = 0.054$). In the group 2 and group 3 analysis, in which the effect of cartilage graft harvesting was more prominent, a very serious loss of tension was again observed ($p = 0.005$). This suggests that both mucoperichondrial dissection and cartilage graft harvesting may have caused a reduction in tension, with an additive effect.

In our study, cartilage strength was evaluated with the axial tension test using the MTS 858 Mini Bionix II universal test machine. Time, axial displacement, and axial force values were recorded as data during the tension test. The curves plotted were used to calculate breaking loads and displacements at the breaking points. There is an issue of whether the test accurately meets the septum physiology and mechanics. Because the septum is cartilage tissue, it is not possible that any type of stress on it will cause damage to itself. Because the septum possesses soft material properties, it will undergo major displacements and will not be damaged under compression, bending, or torsion stresses. Therefore, in our opinion, the most appropriate load type that can damage the septum was tensile force. The tensile strength resulting from the application of tensile force to the septum causes stress on the sections, and therefore the septum is damaged. Thus, in our study, we preferred to apply tensile force as the most appropriate test, considering the septum's material structure, displacement properties, and loading status in its location.

Many valuable clinical comments can be made relating to our study based on the data we obtained. The maintenance of mucoperichondrial integrity, which is known to be important in supplying blood to the septum, should be taken into account at the maximum level. It can be concluded from this study that mucoperichondrial flaps should be dissected unilaterally, and even less, if possible (e.g., when placing a spreader graft, preparation of a small pocket in only that area), as this will positively affect the strength of the remaining septum. In fact, today, many surgeons tend to dissect the septum in a more limited manner rather than bilaterally. This study demonstrates that the classic practice of harvesting grafts by leaving an L-strut septum of 1 cm at the dorsal and caudal areas may have many negative long-term effects. Surgeons should avoid excessive resection of the septum by leaving a certain amount of cartilage (L-strut).

The importance of the septum has become even more prominent when performing a rhinoplasty. It should be kept in mind that the unpredicted loss of rotation and projection and deviations in the nose observed in the long term may have been caused by impairments in the strength and histologic quality of the existing septum. Therefore, in cases that require a large amount of graft, other donor areas such as the ear or rib cartilage or allogenic materials should be considered to prevent weakening of the existing septum.

CONCLUSIONS

Based on our biomechanical analyses, we have demonstrated the negative effects of subperichondromucosal dissection on septum strength. However, the exact histopathologic mechanism is unknown, and further studies at the cellular level are needed. Different combinations and arrangements may be required in addition to the biomechanical tests we applied. Considering the stresses that the human septum may undergo, various biomechanical tests may reveal the loss of strength at a given force. In this way, more detailed negative effects of mucosal dissection on the septum can be determined, which we believe will be quite valuable in helping to prevent complications in patients undergoing septorhinoplasty.

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