European Archives of Oto-Rhino-Laryngology (2019) 276:421–427
https://doi.org/10.1007/s00405-018-5226-7

RHINOLOGY

Histopathological effects of septoplasty techniques on nasal septum mucosa: an experimental study

Semih Ozdemir[1] · Hatice Celik[2] · Ceyhun Cengiz[3] · Naciye Dilara Zeybek[4] · Elham Bahador[4] · Necmi Aslan[2]

Received: 7 August 2018 / Accepted: 28 November 2018 / Published online: 1 December 2018

Abstract

Objective The aim of this study is to evaluate the histopathological effects of septoplasty techniques on the nasal septal mucosa of rabbits with light and electron microscope.

Methods The study was performed on 21 rabbits between August 2016 and February 2017. Rabbits were randomly divided into three groups. In Group-1, while preserving the L-strut structure of the septum, cartilage resection, was performed by open technique septoplasty. In Group-2, the same procedure was done except the resected cartilage was crushed and put back in place. No surgical procedure was performed on the Control group. Postoperative 2nd month; the specimens were histopathologically evaluated by light and electron microscope in terms of changes in the morphology of septum mucosa, perichondrial thickness, cilia and goblet cell deprivation, loss in glands, fibrosis and inflammatory cell infiltration in the lamina propria.

Results The deprivation in cilia, goblet cells, serous gland and increase in the amount of collagen fibers were examined in both Group-1 and 2. The difference in Group-1 and Group-2 were statistically significant in terms of presence of cilia, number of goblet cells and glands and increase in collagen fibers when compared to control ($p < 0.001$, $p = 0.002$, $p = 0.020$, $p = 0.002$, respectively). In terms of perichondrium thickness, statistically significant difference was found between the Control and Group-2 ($p < 0.001$).

Conclusıon In this study, histopathological findings supported that the presence of cartilage in the septum is necessary to prevent the mucosal changes. Long-term studies are needed to observe whether changes in the morphology of epithelium and gland proceed more than 2 months follow-up.

Keywords Nasal mucosa · Nasal septum deviation · Septoplasty · Septal cartilage

This study was presented in the "13th International Congress of Otorhinolaryngology and Head and Neck Surgery, 5–7 April 2017, Ankara, Turkey".

✉ Hatice Celik
haticecelik66@gmail.com

Semih Ozdemir
semih472@hotmail.com

Ceyhun Cengiz
cyhncngz@gmail.com

Naciye Dilara Zeybek
zeybek.dilara@gmail.com

Elham Bahador
Elhambahador@gmail.com

Necmi Aslan
arslan_necmi@yahoo.com

[1] Ankara Gölbaşı Şehit Ahmet Özsoy State Hospital ENT, Bahçelievler Mahallesi, 321. Sk. No: 2, 06830 Gölbaşı, Ankara, Turkey

[2] Otorhinolaryngology Department, Health Sciences University Ankara Training and Research Hospital, Sakarya Mh. Ulucanlar Cd. No: 89, 06230 Altındağ, Ankara, Turkey

[3] Bozok University faculty of Medicine ENT Department, Medrese Mahallesi Adnan Menderes Bulvarı No: 118, 66200 Yozgat, Turkey

[4] Department of Histology and Embryology, Hacettepe University, 06100 Ankara, Turkey

Introduction

Nasal septum deviations are the most common ones among nasal diseases and they are the main cause of nasal obstruction [1, 2]. Surgical treatment is used to fix the anatomical deformities and to improve the declined nasal functions by preserving the mucosa, bone and cartilage structures as much as possible [3–5]. After septoplasty complaints as nasal obstruction, dryness in the nasal passage and crusting may proceed for a long time in a part of patients. In some cases, the feeling of nasal obstruction may proceed permanently. Even though septoplasty is such a frequently practiced surgical procedure, its effects on the septal mucosa have not been investigated yet. The aim of this study is to evaluate the histopathological effects of septoplasty techniques on the nasal septal mucosa of rabbits by light and electron microscope.

Materials and methods

The study was conducted after the approval of Animal Experiments Ethical Committee (date:Aug 16, 2016-decree no: 0034/438). Twenty-one albino New Zealand rabbits, weighing 2.5–3.5 kg. were used. To prevent the growth factors' effect on the study parameters, young adult rabbits aged 14–16 weeks were used. Our study was conducted in accordance with the humane animal use and care rules declared in the Declaration of Helsinki. All of the animals included in the study were sheltered in standard laboratory conditions in which the temperature was 22 ± 2 °C and humidity was stabilized as 60–70% with 12-h periods of daylight and darkness. All of the animals were fed with standard rabbit forage and water. There were no restrictions upon the animals during the entire study. The rabbits were randomly separated into three groups including 7 rabbits in each. The elevation of mucoperichondrial flap and resection of septal cartilage were done by the open technique septoplasty while preserving the L-strut structure of the septum in Group-1. Cartilage resection was performed with the same procedure in Group-2. In this group, the resected cartilage was crushed and replaced. No surgical procedures were done in the Control group.

Surgical technique

The animals were starved one night before experiment. Preoperatively for general anesthesia 50 mg/kg of ketamine hydrochloride (Ketalar, Eczacıbaşı, Turkey) and 5 mg/kg of xylazine (Rompun, Bayer, Germany) were administered intramuscular over the hindlimb. The surgical area over the nasal dorsum was shaved. The anesthesia was applied to the perinasal area, columella and submucoperichondrial areas bilaterally by the injection of 2 ml of lidocaine hydrochloride (20 mg/ml) and epinephrin hydrochloride (0.0125 mg/ml) (Jetokain, Adeka 32 Medicine and Chemical Products A.Ş. Ankara, Turkey).

The sterilization of the operation site was done with Povidone-iodine (*Poviiodeks*® 1000 ml antiseptic solution, KİM-PA Ankara, Turkey). A vertical incision was done over the nasal dorsum skin. The nasal bone was visualized under the skin and subcutaneous tissue. The skin and the periosteum over the nasal bone were elevated by the surgical elevator. The elevation of the upper lateral cartilage from the nasal bone was performed without perforating the mucosa of the upper lateral wall. Nasal bones were lifted by osteotomy with the surgical scissor and then the septum over the nasal dorsum was reached. Mucosa elevation was done with the elevator from the dorsum as well. Nasal septal cartilage was totally exposed. The elevation was done subperichondrially. These procedures were done without any perforations on the mucoperichondrial flaps or septal cartilage.

Approximately, 1×1 cm sized cartilage was resected by preserving the L-strut structure of the nasal septum in Group-1.

In Group-2, the resected 1×1 cm sized cartilage was crushed and this crushed cartilage was put back in place on the septum. The mucoperichondrial flaps were laid over the septum and the lifted nasal bones were put back in place in both groups. The periosteum, subcutaneous tissue and skin were sutured with 4/0vicryl. Nasal tampons were not used in these groups.

All of the rabbits were evaluated in terms of hemorrhage, septal hematoma and infection at the post-operative 72 h and for septal perforation on the second week. At the end of the second month, the rabbits in all groups were killed by administering intravenous 150 mg/kg thiopental sodium (Pental, İ.E. Ulagay Medicine Ankara Türkiye). The nasal septal cartilage was totally excised with the overlying nasal mucosa bilaterally. The specimens were preserved in 10% formaldehyde solution.

Histopathologic analysis

Light microscopic analysis

Nasal mucosa specimens were divided into 2 pieces. One piece of the fresh tissue samples was used for light microscopic analysis. First, they were fixed in 10% formalin solution for 72 h. Then they were dehydrated through a graded ethanol series, cleared in xylene by a tissue processor (Leica TP 1020), and embedded in paraffin blocks. Sections with 5 µm thicknesses were stained with hematoxylin-eosin,

Masson's trichrome and Alcian blue. The samples were examined and photographed using a light microscope(Leica DM6000B, Wetzlar, Germany) with a DC490 digital camera (Leica).

Loss of pseudostratified columnar epithelium and cilia, decrease in the number of goblet cells, the presence of edema, congestion, infiltration of inflammatory cell and degeneration of mucosal glands were evaluated and scored as absent (0), mild (1), moderate (2) or severe (3) by two histologists. The scoring was done semi-quantitatively as in the study of Ercan et al. handled [6]. The number of goblet cells was determined in 10 fields in each section at 40× magnification. The thickness of mucosa, perichondrium and cartilage was measured using the Leica Application Suite image analysis software (Leica, Wetzlar, Germany) and the mean values were determined for each group.

Electron microscopic analysis

The second piece of the nasal tissue samples was used for transmission electron microscopic analysis. First, tissue samples were fixed in 2.5% glutaraldehyde solution in phosphate buffer, pH 7.4 for 4 h. Then samples were post-fixed for 1 h in 1% osmium tetroxide solution in 0.1M phosphate buffer. After washing in phosphate buffer, they were dehydrated in a graded series of ethanol to absolute ethanol, treated with propylene oxide and embedded in Araldite/Epon812 (EMS, Cat No:13940, PA, USA). After heat polymerization, ultrathin sections (Leica ultracut R Wetzlar-Germany) were double-stained with uranyl acetate and lead citrate (Leica EM AC20). These sections were examined in JEOL-JEM 1400 electron microscope and photographed by Gatan orius CCD camera (USA).

Statistical analysis

The analysis of data was done on the package program IBM SPSS Statistic 17.0 (IBM Corporation, Armonk, NY, USA). The Shapiro–Wilk test was used to determine whether the distribution of continuous numerical variables was close to normal, and the homogeneity of variances was investigated using the Levene test. Descriptive statistics were expressed as mean ± standard deviation or median (minimum to maximum) for continuous numerical variables, while they were expressed in terms of number of observations and percentages for sequential variables.

The significance of difference between means of samples was tested with Student's *t* test when there were two independent samples. On the other hand, when there were more than two independent samples, it was tested with one-way ANOVA. The significance of difference with respect to deviation from normal or sequential variables between samples was tested with Mann–Whitney *U* test when there were two independent samples. When there were more than two independent samples Kruskal–Wallis was used. When the one-way ANOVA or Kruskal–Wallis test results were significant, the sample(s) causing the difference was determined using the post-hoc Tukey HSD or Bonferroni correction Mann–Whitney *U* test.

The results for $p < 0.05$ were considered statistically significant. However, in all possible multiple comparisons made with non-parametric test statistics, the Bonferroni correction was made to control the Type I error.

Results

Histopathological findings of the Control group

Hyaline cartilage and on either side, lamina propria containing glands, blood vessels, nerve fibers and overlying pseudostratified columnar epithelium with goblet cells showed regular morphology in the nasal septum of Control group (Fig. 1).

In transmission electron microscopy, the apical surface of epithelial cells was highly specialized with cilia and microvilli in the control group (Fig. 2a). The tight junctions between epithelial cells were observed at the apical domain of the epithelial cells in the Control group.

The average score for this group's specimens in terms of cilia loss, decrease in the number of goblet cells, degeneration in mucosal glands, congestion, fibrosis in the lamina propria, edema and inflammatory cell infiltration was determined to be 0.

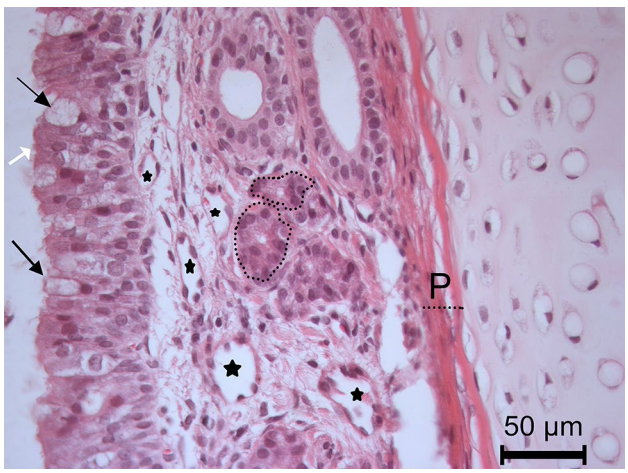

Fig. 1 Nasal septum sample from Control group. Perichondrium (P), chondroblasts, chondrocytes are observed in hyaline cartilage, and blood vessels (star), gland sections (dotted line) are observed inside the connective tissue under pseudostratified columnar ciliated (white arrow) epithelium with goblet cells (black arrow) (Hematoxylin-eosin ×400)

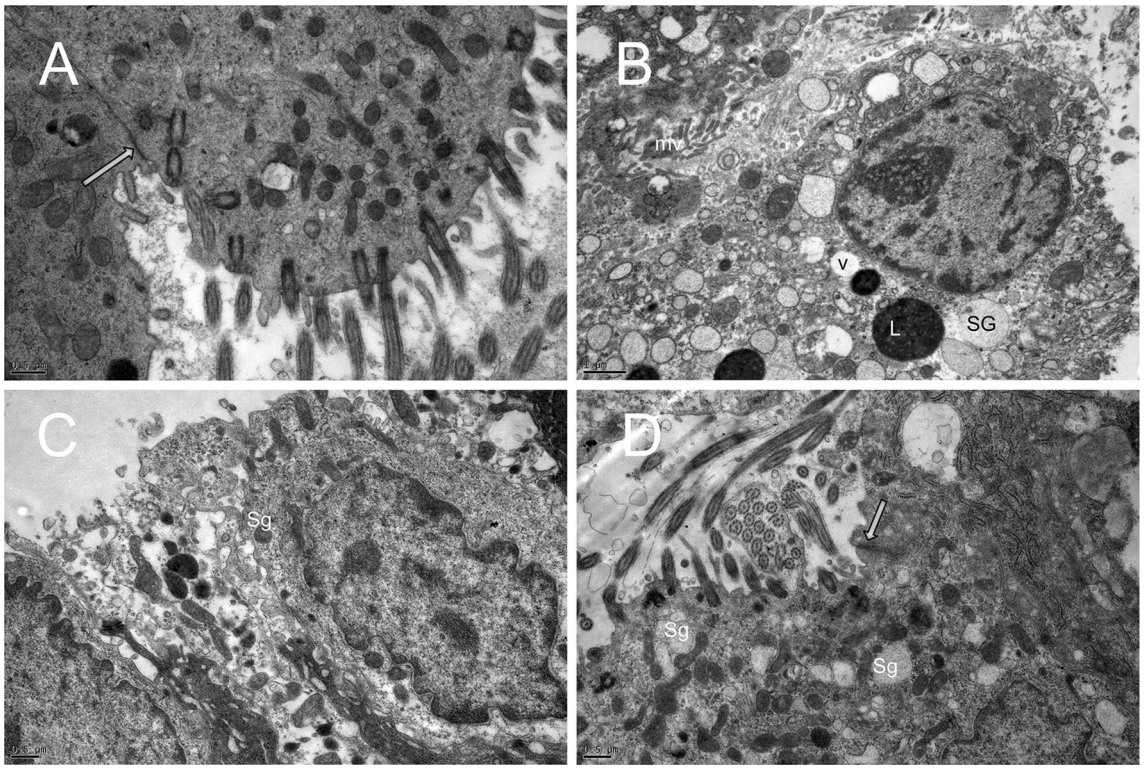

Fig. 2 Transmission electron micrographs of the epithelium. **a** Epithelial cells with cilia and microvilli. The tight junctions (arrow) between epithelial cells in the Control group. **b** Severe loss of cilia and vacuoles (V), lysosomes (L), secretory granules (SG) in the epithelial cell in Group-1. **c** Epithelial cells with loss of cilia in Group-2. **d** Epithelial cells with cilia in Group-2 (Uranyl acetate-Lead citrate, **a** ×25000, **b** ×15000, **c, d** ×20000)

Histopathological findings of the Group-1

The cartilage tissue was not observed in 6 specimens of Group-1. Simple cuboidal epithelium was observed and Goblet cells were missing in these samples. The glands were present in the connective tissue. Loss of serous glands, edema, congestion, inflammatory cell infiltration was minimal. In lamina propria collagen fibers were increased moderately (Fig. 3).

Transmission electron microscopy revealed severe loss of cilia in the epithelium of Group-1. The vacuoles, lysosomes, secretory granules were observed in the epithelial cells of Group-1. In some areas the microvilli were present. The intercellular area was filled with degenerated cell particles (Fig. 2b).

In this group according to the average scoring, severe cilia loss, decrease in the number of goblet cells, mild edema, mild degeneration in mucosal glands, mild congestion and mild inflammatory cell infiltration with moderate increase in collagen (fibrosis in lamina propria) was determined.

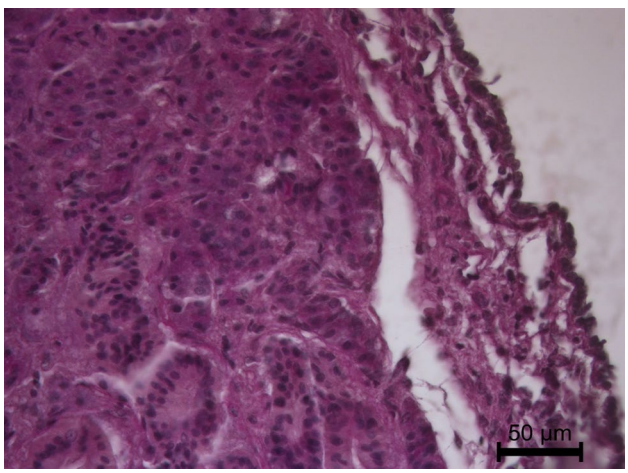

Fig. 3 In the specimen from Group-1, a simple cuboidal epithelium, which replaces pseudostratified columnar ciliated epithelium with goblet cells is observed partly. No goblet cells are observed (PAS-Alcian blue ×400)

Histopathological findings of the Group-2

In Group-2 proliferation of chondrocytes, increase in the thickness of the cartilage and the perichondrium was

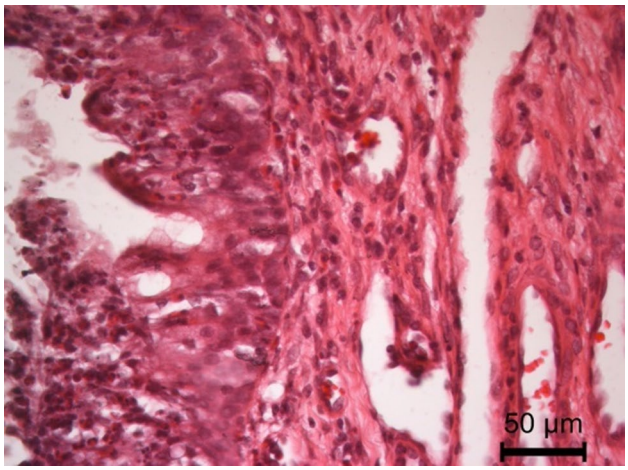

Fig. 4 Goblet cell loss and impairment of epithelium are observed in the specimen from Group-2. Inflammatory cell infiltration is observed in the epithelium and in the underlying connective tissue (Hematoxylin-eosin ×400)

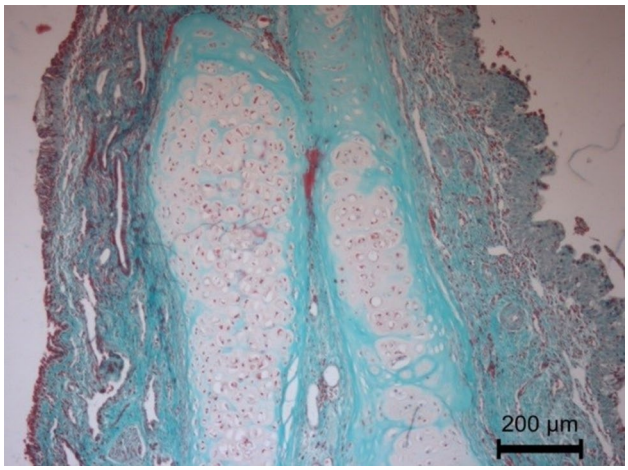

Fig. 5 Nasal septum sample from Group 2. The crushed cartilage is seen overlapping the existing cartilage in the tissue. Thicker perichondrium in the immature cartilage and also in the edge of existing cartilage. Empty lacunas lacking chondrocyte are observed in the crushed parts of the crushed cartilage (Masson trichrome ×200)

detected. The epithelium was preserved in one side. However, ciliated epithelium and Goblet cells were not observed on the other side (Fig. 4). The glands were missing in the areas where the cartilage was thicker. The crushed cartilage tissue was observed over the existing cartilage tissue in some samples of Group-2. The thickness of the perichondrium and the number of chondroblasts were increased in these samples (Fig. 5).

In this group, while moderate loss of cilia, goblet cell and serous gland were observed, edema and congestion were not detected. Mild increase in collagen fibers in lamina propria and severe infiltration of inflammatory cells were detected.

Transmission electron microscopy supported the loss of cilia in some areas of epithelium in Group-2 (Fig. 2c, d).

Statistical findings

The scores for loss of cilia were higher in Group-1 and Group-2. There was a statistically significant difference between these groups and control in the aspect of cilia loss ($p < 0.001$). No statistically significant difference was observed among groups in terms of edema scores ($p = 0.067$). Statistically significant difference was observed in scores for loss of serous gland ($p = 0.020$) (Table 1). The scores for increase in collagen fibers of lamina propria in Group-1 and Group-2 were higher and a statistically significant difference was found between these groups and control ($p = 0.002$ and $p = 0.002$). No statistically significant difference was obtained in aspect of the inflammation ($p = 0.097$) (Table 1).

The statistical results of the differences between groups in terms of mucosal thickness and goblet cell count are presented in Table 2.

The difference in terms of perichondrium thickness between the Control and Group-2 was found statistically significant ($p < 0.001$). In the comparison of the Control and Group-2 in terms of nasal septum thickness, statistically significant difference was not found ($p = 0.641$) (Table 3).

Complications

The complications as infection, septal hematoma and septal perforation were not seen in any of the rabbits.

Discussion

Nasal septum deviation, which prevents nasal air-flow is one of the most common reasons of nasal obstruction [6, 7]. It may cause crustering, drying and bleeding. Septoplasty can be performed with different techniques. In some patients whose nasal deviation is treated after septoplasty might still have various degrees of complaints as nasal obstruction and dryness in the mucosa. Mucosal alterations seen after septoplasty is still a question. Studies evaluating the effects of different septoplasty techniques on nasal septal mucosa are limited in the literature. The aim of this study is to evaluate the effects of septoplasty techniques on the nasal septal mucosa in rabbits.

Septal mucosa consists of ciliated and non-ciliated cells, goblet cells, seromucous glands, lamina propria and perichondrium. Ciliated cells are important for providing mucociliary transport. Seromucous glands secrete nasal mucous. Goblet cells produce glycoproteins, which provide viscous and elastic feature of nasal mucous. Changes occurring in

Table 1 Frequency distributions of the observations in terms of histopathological scores according to the groups

	Control	Group-1	Group-2	*P* value[†]
Ciliary loss				< 0.001
Absent	6[a,b]	0	0	
Mild	0	0	1	
Moderate	1	1	2	
Severe	0	6[a]	4[b]	
Edema				0.067
Absent	4	1	5	
Mild	3	5	2	
Moderate	0	1	0	
Severe	0	0	0	
Serous gland loss				0.020
Absent	7[b]	4	1	
Mild	0	0	3	
Moderate	0	0	1	
Severe	0	3	2	
Vascular congestion				0.417
Absent	4	4	6	
Mild	3	2	1	
Moderate	0	1	0	
Severe	0	0	0	
Collagen fibril increase in lamina propria				0.002
Absent	6[a,b]	0	0	
Mild	1	4	5[b]	
Moderate	0	2	2	
Severe	0	1	0	
Inflammation				0.097
Absent	4	4	1	
Mild	3	0	1	
Moderate	0	1	3	
Severe	0	2	2	

[†]Kruskal–Wallis test

[a]The difference between the Control Group and Group-1 is statistically significant ($p < 0.001$)

[b]The difference between the Control Group and Group-2 is statistically significant ($p < 0.01$)

seromucous glands affect the quantity and viscosity of nasal mucous [8].

Degraded mucociliary transport, increase in inflammatory cells, loss in cilia, decrease in seromucous glands were reported in studies evaluating mucociliary clearance, histological features and surface structure of nasal septum in patients with septal deviation [9, 10].

Structural changes in goblet cells and submucosal glands after partial or extended mucosal sinus resection are evaluated in an animal study [11]. In this study, an increase in goblet cell density and a decrease in the number of submucosal glands were reported after extended resection of the maxillary sinus mucosa. An increase in mucus viscosity and a decrease in mucus production were observed. This mentioned findings suggested to have a role in continuing complaints as crusting and obstruction postoperatively.

Histological changes in maxillary sinus mucosa after functional endoscopic sinus surgery (FESS) in patients with chronic sinusitis were evaluated in a clinical study with their biopsy specimens taken from the maxillary sinus wall [12]. The patients were reassessed after one year postoperatively and complete healing was not observed including asymptomatic patients.

In our study, less goblet cells were found in the totally resected-cartilage group. No statistically significant difference was found between the control and the group in which cartilage was re-implanted after crushing. Severe serous gland loss was reported in three of the rabbits having totally cartilage resection, whereas two of the rabbits in which cartilage was reimplanted after crushing. The loss in cilia was more severe in Group-1 than Group-2. Intensive collagen fiber augmentation (fibrosis) was observed in one rabbit in the totally resected-cartilage group. All these findings pointed that mucosal support due to existing cartilage preserves goblet cells, however, crushed cartilage can induce perichondrium reaction and submucosal glands may be negatively effected as a result of accelerated fibrosis.

The thickness of the perichondrium could not be evaluated in Group-1 as the cartilage was totally resected. Therefore, perichondrial thickening was statistically significant in the re-implanted group when compared to control ($p < 0.001$). This may be related to the induction of perichondrium reaction due to the mechanical damage to the cartilage.

The nasal mucosa was anatomically changed by rotation flaps in a study and mucociliary transport from the proximal side to distal side were evaluated [13]. At first week, first month and ninth month, mucociliary activity direction and histopathological changes of mucosal flaps were evaluated. Mucociliary transport direction was reported to be from distal to proximal again and histopathological changes were recovered to preoperative period.

The wound healing and histomorphological changes of nasal septal mucosa were evaluated in mechanically damaged rat mucosa [14]. The specimens were evaluated in terms of inflammatory cell infiltration, goblet cell count and cilia formation. Additionally, subepithelium and epithelium thickness (STI and ETI) indexes were measured. Fibrosis was evaluated with the subepithelial fibrosis index (SFI). Consequently, the healing process after mechanical injury in rats was experimentally observed and it was reported that on the 28th day the respiratory mucosal regeneration was completed.

Table 2 Thickness of mucosa and number of goblet cells according to the groups

	Average	Std. deviation	Minimum	Maximum	*P* value[†]
Thickness of mucosa					0.788
Control	502.76	236.98	250.60	953.60	
Group-1	549.50	122.98	392.88	716.83	
Group-2	482.51	177.08	233.86	738.52	
Number of goblet cells					0.004
Control[a]	8.84	0.63	8.00	9.70	
Group-1[a]	2.03	3.46	0.00	9.10	
Group-2	4.56	3.32	0.00	10.10	

[†]One-way variance analysis (One-Way ANOVA)

[a]The difference between the Control Group and Group-1 is statistically significant ($p = 0.003$)

Table 3 Measurements of thickness of perichondrium and nasal septum according to the groups

	Control	Group-2	*P* value
Thickness of perichondrium	28.83(22.58–39.00)	97.07(38.27–173.72)	$< 0.001^{\dagger}$
Nasal septum	354.83 ± 139.80	320.28 ± 130.57	$0.641^{\ddagger}$

[‡]Student's *t* test

[†]Mann–Whitney *U* test

In our study, acute inflammatory process of nasal mucosa after septoplasty improved. In one rabbit of Group-1, in which cartilage was totally resected, severe collagen fiber increase in lamina propria was observed. This finding may be attributed as a chronic change, but it is not a certain sign of chronic inflammatory process after septoplasty in which cartilage support is not protected. All these findings are 2 months follow-up results of an experimental animal study. For the correlation of histopathological and clinical results of different septoplasty techniques, human studies are needed.

Conclusion

In this study, histopathological findings supported that the presence of cartilage in the septum is necessary to prevent the mucosal changes. Perichondrial thickening in the cartilage-crushed group may be a result of perichondrium reaction due to mechanical damage. Long-term studies such as more than 2 months follow-up are needed to observe the changes in the epithelium and glands in lamina propria.

Compliance with ethical standards

Conflict of interest The authors do not have any conflicts of interest. No financial support was obtained for this study.

Ethical approval The study was conducted after the approval of Animal Experiments Ethical Committee of our Hospital (date:Aug 16, 2016-decree no: 0034/438).

References

1. Most SP, Rudy SF (2017) Septoplasty: basic and advanced techniques. Facial Plast Surg Clin 25:161-
2. Hwang PH, McLaughlin RB, Lanza DC, Kennedy DW (1999) Endoscopic septoplasty: indications, technique, and results. Otolaryngol Head Neck Surg 120:678–682
3. Rosen CA, Johnson JT (2014) Bailey's head and neck surgery otolaryngology review, 1st edn. Lippincott Williams and Wilkins, Baltimore
4. Sedaghat AR, Busaba NY, Cunningham MJ, Kieff D (2013) Clinical assessment is an accurate predictor of which patients will need septoplasty. Laryngoscope 123:48–52
5. Sundh C, Sunnergren O (2015) Long-term symptom relief after septoplasty. Eur Arch Otorhinolaryngol 272:2871–2875
6. Tsang CLN, Nguyen T, Sivesind T, Cervin A (2018) Long-term patient-related outcome measures of septoplasty: a systematic review. Eur Arch Otorhinolaryngol 275:1039–1048
7. Türk B, Akpinar M, Altundağ A, Kirik M, Ünsal Ö, Coşkun BU (2017) The effect of external approach septoplasty on olfactory function. J Craniofacial Surg 28:1675–1678
8. Huizing EH, De Groot JW (2003) Functiona reconstructive nasal surgery, 1st edn. Thieme, New York
9. Jang YJ, Myong NH, Park KH, Koo TW, Kim HG (2002) Mucociliary transport and histologic characteristics of the mucosa of deviated nasal septum. Arch Otolaryngol Head Neck Surg 128:421–424
10. Mariappan RG, Dhanalakshmiv M, Mathaikutty DM, Shanmugam R, Shanmugam VU, Swaminathan B, Nandipati S (2014) Clinicopathological correlation and the effect of septal surgery on nasal mucociliary clearance. Sch J App Med Sci 2:1691–1695
11. Melgarejo Moreno P, Hellín Meseguer D (2006) Submucosal glands and goblet cells in maxillary sinus surgery: an experimental study in rabbits. Rhinology 44:259–263
12. Anselmo-Lima WT, Ferreira MD, Valera FC, Rossato M, de Mello VR, Demarco RC (2007) Histological evaluation of maxillary sinus mucosa after functional endoscopic sinus surgery. Am J Rhinol Allergy 21:719–724
13. Ateşpare A, Üstündağ E, Dalçık H, Çelik Ö (2015) Mucociliary transport and histopathological changes in rotation flaps of the nasal mucosa. Eur Arch Otorhinolaryngol 272:1143–1148
14. Khalmuratova R, Jeon SY, Kim DW, Kim JP, Ahn SK, Park JJ, Hur DG (2009) Wound healing of nasal mucosa in a rat. Am J Rhinol Allergy 23:33–37