

Microbial biofilm formation on silicone nasal splints: optimal time for splint removal*

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Abstract

Objectives: Biofilms are sessile communities associated with persistent infections and are resistant to conventional therapeutic strategies. They survive on the surface of various inorganic medical devices and cause serious medical problems.

Methods: We recruited 25 patients who underwent nasal surgery between January and May 2013. All patients received silicone splints at the conclusion of the procedure. Pieces of the splints were collected 48, 72 and 96 h post-surgery and prepared for scanning electron microscopy evaluation to assess biofilm formation.

Results: Biofilm was observed in 3, 14 and 25 of the 25 samples at 48, 72 and 96 h, respectively. The differences in the proportions of the samples with biofilm formation at each time point (48, 72, and 96 h) were statistically significant.

Conclusion: Our data demonstrated that biofilm formation on silicone splints increases significantly after 48 h following placement. Although packing may reduce complications, surgeons must consider the potential hazards of packing materials, such as biofilm formation at 48 h post-surgery.

Key words: biofilm, scanning electron microscopy, nasal splints, antibiotics, complications

Introduction

Nasal septal surgery is a common otorhinolaryngological procedure and may be performed separately or as part of a surgical plan⁽¹⁾. As with most surgical procedures, septoplasty has potential complications which include the formation of synechiae, bleeding and septal haematoma⁽²⁾. Various intraoperative and postoperative measures have been developed to prevent these complications, including proper subperichondrial elevation, transseptal fixation sutures and postoperative nasal packing. Of these measures, nasal packing is the most controversial. Several complications have been reported following nasal packing including dysphagia, aspiration, airway obstruction, hypoventilation, hypoxemia, local and/or general infections, the feeling of

discomfort and reduced quality of life^(3,4).

Antibiotic ointment applied to the packing material and prophylactic enteral or parenteral antibiotics have been used to prevent nasal packing-related infections. However, local or general infection-related complications, such as vestibulitis, cellulitis, septal abscess, sinusitis, meningitis, cavernous sinus thrombosis and intracranial abscess may occur despite the use of preventive strategies⁽⁵⁾. Most surgeons recommend not using packing if possible; however, when the procedure is necessary, there are no objective data to provide guidelines regarding the optimal removal time. Several surgeons strongly recommend removing the nasal packs in a timely fashion to reduce infection-related problems following nasal septal surgery. A recent report

suggests that silicone sheets that sit on both sides of the septum to hold it straight and that are left in place for up to 96 h are useful for preventing postoperative complications ⁽⁶⁾.

Since the relationship between microbial biofilms and the development of various human infections was revealed ⁽⁷⁾, clinicians have been mindful of the role played by biofilms in the aetiology of microbiological manifestations. Biofilm communities are unique and highly resistant to standard antibiotic therapies. They have evolved several mechanisms of antibiotic resistance distinct from those of planktonic bacteria. We measured biofilm formation on silicone nasal splints used at the conclusion of nasal septal surgery to determine the optimal timeframe for the removal of nasal packing material.

Materials and methods

Our prospective, nonrandomised controlled study included 25 consecutive patients with nasal septal deviation and a history of nasal obstruction that were scheduled to undergo nasal septoplasty at Eskisehir Yunus Emre State Hospital ENT Department. The ethics committee of Eskisehir Osmangazi University approved the study protocol, and all participants provided informed consent. Standard physical examinations with anterior rhinoscopy and rigid nasal endoscopy were performed, and patients who had concurrent sinusitis, allergic rhinitis, or nasal polyposis were excluded from the study.

Surgery

Surgical procedures were performed under local anaesthesia by the same surgeon. All participants were premedicated using 10 mg intramuscular diltiazem hydrochloride and received 1 g intravenous co-amoxiclav for antimicrobial prophylaxis. Gauzes soaked in xylometazoline 0.1% were used for nasal mucosal decongestion. We injected 2-mL lidocaine HCl 1% with adrenaline 1:100,000 to achieve vascular haemostasis. Briefly, our approach involved a Killian incision, creation of subperichondrial tunnels, posterior chondrotomy, osteotomy if necessary and septal reconstruction. Nasal packing using silicone splints that contained no lubricant agent or topical antimicrobial was performed at the conclusion of surgery. One silicone splint was applied to each nasal cavity and left in place for 96 h. In general, nasal splint removal times vary widely, usually depending on the surgeon and the patients' status. We removed the splints at 96 h to allow us to compare biofilm formation at three time points (48, 72 and 96 h).

Sample collection

Patients were hospitalised for 24 h and were administered co-amoxiclav (2 g per day orally). The first samples were collected under sterile conditions from the most caudal end of the splints 48 h after placement. The second and third samples were

under taken under sterile conditions at 72 and 96 h, respectively. All samples were immediately prepared for scanning electron microscopy evaluation.

Scanning electron microscopy

The silicone samples were immediately placed in 2.5% glutaraldehyde (prepared in 0.1 M phosphate buffer, pH 7.4 for 24 h at 4°C as a prefixation step). The samples were rinsed twice with 0.1 M phosphate buffer (pH 7.4) and postfixed in 1% osmium tetroxide for 1 h at room temperature and then rinsed again with distilled water. Following that, the specimens were dehydrated in graduated concentrations of ethyl alcohol (30, 50, 70, 90 and 96%) for 15 min each followed by absolute alcohol for 30 min. After that, the specimens were dried using a Critical Point Dryer (Polaron CPD, Quorum Technologies, East Sussex, UK). Carbon conductive paint and gold coat (Polaron SC762-Sputter Coater, Quorum Technologies) were used for mounting and specimen coating, respectively. Finally, each specimen was examined using a JEOL scanning electron microscope (JEOL JSM05600LV, Jeol Ltd., Tokyo, Japan). The surface of each sample was scanned systematically. We defined biofilm architecture as dense accumulations of microorganisms within an amorphous matrix, according to Chole and Faddis ⁽⁸⁾.

Statistical analyses

We used t-tests to detect significant differences in the ratio of biofilm formation between time periods.

Results

Of the 25 patients, 16 were male and 9 were female, and the age range of participants was 24–60 years (mean age, 36.3 years).

The SEM findings revealed that 3 (12%), 14 (56%) and 25 (100%) of the 25 silicone samples exhibited microbial biofilm formation on their surface at 48, 72 and 96 h, respectively (Figures 1,2, 3 and 4; Table 1). The percentage of biofilm formation was significantly different between the 48- and 72-h, between 48 and 96 h and between the 72- and 96-h time points ($p < 0.001$ for both). No intra- or postoperative complications occurred.

Discussion

Nasal septal procedures may conclude with nasal packing, and various types of materials have been used for this task. Although these endonasal materials play an important role during the postoperative period by preventing bleeding and septal haematoma formation, they are synthetic and "foreign" objects in the body. As with all foreign materials, nasal packs may cause infection. Otherwise, nasal airway procedures are considered to be contaminated and may contribute to postoperative infectious complications ⁽⁹⁾. The risk of infection is the primary reason for use of postoperative prophylactic antibiotics.

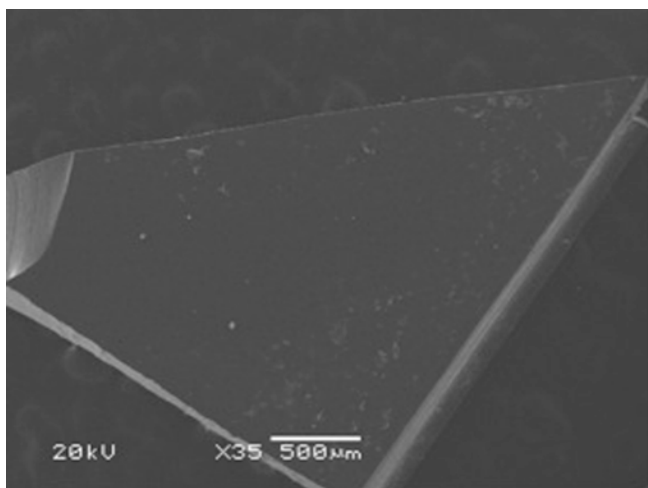


Figure 1. Scanning electron micrograph of a silicone splint at low (X35) magnification (48 h).

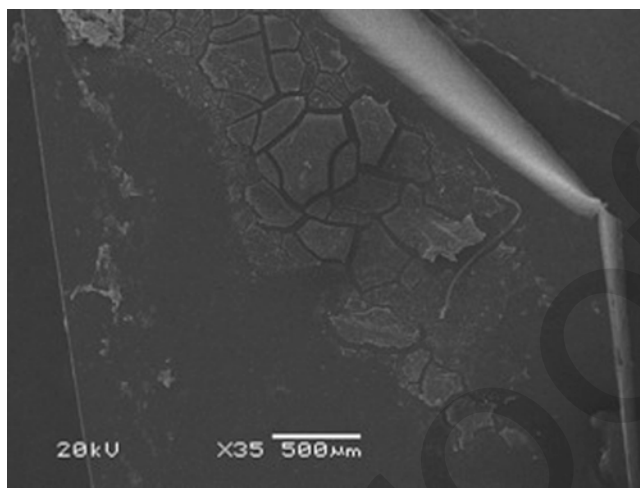


Figure 3. Scanning electron micrograph of a silicone splint with biofilm at low (X35) magnification (96 h).

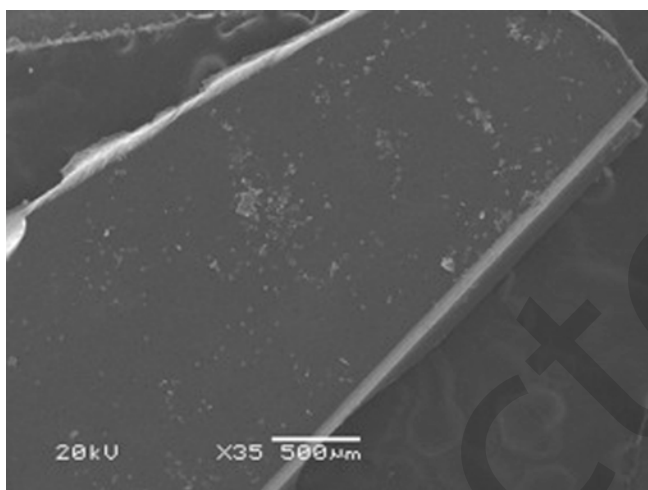


Figure 2. Scanning electron micrograph of a silicone splint with biofilm at low (X35) magnification (72 h).

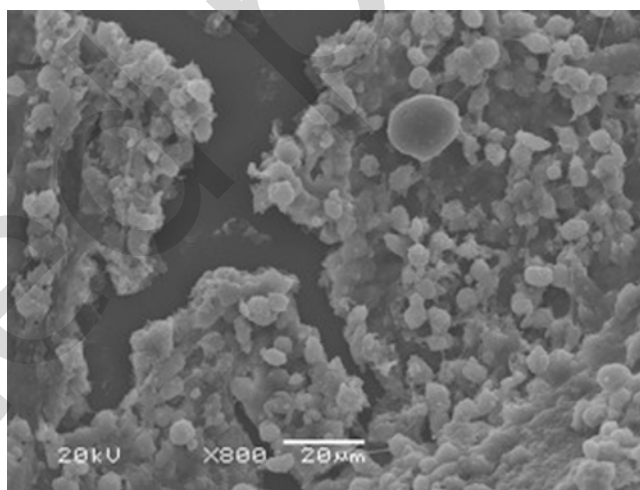


Figure 4. Scanning electron micrograph of a silicone splint with biofilm at high (X800) magnification (96 h).

According to Rechtweg et al. ⁽¹⁰⁾, the most common reasons for prophylactic antibiotic use following rhinological procedures are to prevent postoperative infection (60.4%), avoid toxic shock syndrome (31.5%) and to avert medicolegal issues (4.9%). We used prophylactic antibiotics in our study to prevent infection. Furthermore, guidelines for the optimal time to remove nasal packing are critical because bacteraemia increases within 48 h of placement ⁽⁵⁾, and patients may experience significant discomfort when packing is left in place for 2 or more days ⁽¹⁾. We left the silicone splints in place for 96 h, which is a common timeframe in general practice; however, no randomised clinical trials have investigated the optimal time for the removal of nasal packing. Surgeons are divided on the efficacy of nasal packing; some argue that it is not necessary and septal suturing achieves the same goal ⁽¹¹⁾, others continue to use nasal packing to

prevent potential complications. Nonetheless, nasal packs left in place for more than 48 h may be uncomfortable for the patient and increase the risk of infection. As a personal preference of the senior author of the present study, we used a silicone splint as the packing material. Although postoperative synechiae formation did not occur in our patient group, this finding may not be considered an advantage of silicone splints because most randomised clinical trials have shown that silicone splints cause a significant increase in postoperative pain with no sufficient evidence of a decrease in the rates of intranasal adhesions or other clinically significant complications ^(12,13). Our findings in silicone nasal splints are consistent with those of previous studies showing that the rate of biofilm formation in nasal packing increases after 48 h. Biofilm cells are physiologically distinct from those existing planktonically on several

Table 1. Patients and SEM findings.

Patient No & Gender	Age	Time Period (Hour)		
		48	72	96
1 : F	39	-	-	+
2 : F	29	-	-	+
3 : M	33	-	-	+
4 : M	37	-	+	+
5 : F	45	-	-	+
6 : F	35	-	+	+
7 : M	27	-	+	+
8 : M	46	-	+	+
9 : M	24	+	+	+
10 : F	28	-	+	+
11 : M	28	-	-	+
12 : M	29	-	-	+
13 : M	58	-	-	+
14 : F	37	-	+	+
15 : F	54	-	+	+
16 : M	60	-	-	+
17 : M	41	+	+	+
18 : M	35		+	+
19 : M	49		+	+
20 : F	36		-	+
21 : M	24	+	+	+
22 : M	28		+	+
23 : M	26		-	+
24 : F	33		-	+
25 : M	27		+	+

* M: Male, F: Female

** (-): No biofilm formation, (+): Biofilm formation

levels, such as gene transcription, phenotype and resistance to antibacterial agents^(14,15). The high level of antibiotic resistance exhibited by biofilm occurs via several mechanisms, including (i) decreased penetration or diffusion of antimicrobial agents into biofilms, (ii) increased activity of multidrug efflux pumps, (iii) involvement of quorum-sensing systems, (iv) starvation or stress responses and (v) genetic switches that transform susceptible planktonic cells into antibiotic-resistant persisters⁽¹⁶⁾. Strategies to eradicate biofilm are limited. The most effective method for removing biofilm is excision of the affected tissue or foreign material at the optimal time⁽⁸⁾. Our findings show that despite antibiotic prophylaxis, infection-related complications may occur at 48 h following the placement of nasal silicone splints, and infection is likely to occur at 96 h following packing. Our findings are consistent with previous reports that 48 h is the optimal time for packing removal to prevent infection-related complications and minimise patient discomfort. The fact that none of our patients experienced postoperative complications limits our ability to draw conclusions regarding the relationship between biofilm formation and the risk of postoperative complications. Thus, more studies of the association between biofilm and postoperative complications are needed.

In conclusion, our data showed that biofilm formation on the surface of nasal silicone splints increases significantly after 48 h. Although postoperative interventions, such as nasal packing with silicone splints, may reduce the frequency of surgical complications, surgeons must be aware of the potential hazards, such as biofilm formation 48 h after application.

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Authorship contribution

MA: Surgical procedure; TÇ: Manuscript writing; ID: SEM study; TS: Data collection, analysis; CC: Data control, Revised manuscript

Dr. M. Acar takes responsibility for the integrity of the content of the paper.

Conflicts of Interest

Authors declare that there is no competing interest.

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