Hacettepe Dişhekimliği Fakültesi Dergisi
Cilt: 31, Sayı: 1, Sayfa: 12-18, 2007

Effects of 0.2% Chlorhexidine Gluconate to the Plaque Accumulation on Silk Suture Materials in Oral Mucosa: A Scanning Electron Microscope Study

Ağız Mukozasında İpek Sütur Materyalleri Üzerinde Plak Birikimine %2'lik Klorhekzidin Glukonatın Etkileri: Scanning Electron Mikroskop Çalışması

***Ebru OLGUN ERDEMİR DDS, PhD, **Umut SARACOĞLU TEKİN DDS, PhD, ***Ali ERDEMİR DDS, PhD, ****Selcuk AKTÜRK PhD**

* Department of Periodontology, Faculty of Dentistry, University of Kırıkkale
** Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Kırıkkale
*** Department of Endodontics, Faculty of Dentistry, University of Kırıkkale
**** Department of Physics, Faculty of Science and Art, University of Kırıkkale

ABSTRACT

Aim: To evaluate the effect of chlorhexidine to the plaque accumulation on silk suture by scanning electron microscope (SEM).

Materials and Methods: The study included fourteen subjects. The patients were randomly divided into two groups. Group 1st, the test group, used a % 0.2 chlorhexidine gluconate; Group 2nd, the control group, used saline after the impacted third molars extraction. The sutures were removed after 7 days and kept in 70% alcohol and processed for SEM. The material was critical-point dried using dry ice method and observed in a field emission SEM at different magnifications. The contamination of the suture surfaces was scored and the Mann-Whitney U test was used for statistical analysis.

Results: There were obvious differences in the amount of plaque and debris accumulated between the test and the control groups. The amount of contamination was significantly lower in the test group ($P<0.01$).

Conclusion: SEM observation showed that the test group had less contaminated surface area than the control group. The results indicate that CHX was effective in the reduction of plaque accumulation on silk suture.

KEYWORDS
Chlorhexidine gluconate, Dental plaque, Silk sutures, SEM

ÖZET

Amaç: Klorhekzidin glukonatın ipek sütur materyallerinde plak birikimi üzerine etkisini SEM ile değerlendirmektir.

Gereç ve Yöntem: Çalışmaya 14 kişi katılmıştır. Hastalar rasgele iki gruba ayrılmıştır. Gömülü 3. molar operasyonundan sonra, birinci grup, test grubu, % 0.2'lik klorhexidin glukonat kullandı; ikinci grup, kontrol grubu, serum fizyolojik kullandı. Sütur materyalleri 7 gün sonra alındı ve SEM işlemi için % 70'lik alkolde saklandı. Materyal dry-ice yöntemi kullanılarak kritik noktada kurutuldu. Sütur yüzeylerinin kontaminasyonu skorlandı ve istatistiksel analiz için Mann-Whitney U testi kullanıldı.

Bulgular: Test ve kontrol grupları arasında plak ve debris miktarında belirgin farklılıklar olduğu gözlendi. Kontaminasyon miktarı, test grubunda belirgin şekilde daha azdı ($P<0.01$).

Sonuç: SEM gözlemi test grubunun kontrol grubundan daha az kontamine olmuş yüzeye sahip olduğunu göstermiştir. Sonuçlar, klorhekzidinin ipek sütur materyali üzerine plak birikiminde azalmaya neden olduğunu ortaya koymuştur.

ANAHTAR KELİMELER
Klorhexidin glukonat, Dental plak, İpek sütur materyalleri, SEM

INTRODUCTION

Mouth rinses are a common adjunct to mechanical hygiene measures to facilitate the control of supragingival plaque, therefore dental caries, gingivitis and postoperative infection[1-3]. Chlorhexidine gluconate (0.2%) (CHX) has proven in many studies to be the most efficient solution and is still called the gold standard.[4] Many indications for the use of this antiseptic have been proposed. One of the most important usages is the secondary prevention after oral surgery including periodontal therapy, root-end resection and third molar surgery[5-7].

CHX is a bisbiguanide antiseptic active against gram-positive and gram-negative bacteria, facultative anaerobes and aerobes, moulds, yeasts and viruses. Oral CHX mouth rinses have been effective in decreasing plaque formation and controlling gingivitis[8,9] and dental caries.[10,11] Its antibacterial activity arises from its positive charge at physiological pH, which produces nonspecific binding to the negatively charged membrane phospholipids of bacteria; this causes an alteration in bacterial osmotic equilibrium, with potassium and phosphorus leakage. More recently, CHX has been reported to inhibit the activities of two types of matrix metalloproteinases (gelatinases A and B) via a cation-chelating mechanism[12]. The final inhibitory action of CHX on plaque formation on teeth may occur via different mechanisms[13,14] (i) immediate bactericidal effect, (ii) prolonged bacteriostatic effect by surface-bound CHX, (iii) blockage of the acidic groups from the salivary glycoproteins that form the pedicle, (iv) binding to the bacterial surface in sublethal amounts so that initial adhesion to the surface is inhibited, (v) disturbing the plaque formation by precipitation of agglutination factors in saliva and by displacing calcium from the plaque matrix.

Organic and synthetic non-resorbable and resorbable suture materials are currently used in surgery within the mouth[15-17].

Silk has been a favored suture material in oral, periodontal and endodontic surgery because of its ease of handling[15]. However many studies have reported that silk causes a more intense and prolonged inflammatory response in gingival and oral mucosa than synthetic materials[15,18,19].

The tendency for microbial attachment and accumulation on suture materials is one of the most important problem in the healing period. Bacteria and debris that attach or lodge between the suture materials could delay repair and maintain infection[16,17].

Thus, this clinical trial aimed to examine the anti-plaque effectiveness of CHX on silk suture materials with SEM.

MATERIAL AND METHODS

The study population included 14 patients, in the age range of 1st group 20-36 years (26.1±5.3) and in the age range of 2nd group 23-43 years (29.8±7.9). The patients had gingivitis as evidenced by multiple sites with a probing depth of 3 mm or less and without bone loss by radiographs. All participants were periodontally untreated and had not previously received surgical therapy and were drawn from the patients with gingivitis at the Department of Periodontology. All subjects were systemically healthy, with no medical condition that would effect their participation in the study. All of them have totally or partially bone-impacted mandibular third molar. Exclusion criteria applied were a course of anti-inflammatory or antimicrobial therapy within the previous 3 months, a history of regular use of mouthwashes, and had mucosal lesions. All participants received primary phase of non-surgical treatment including oral hygiene instruction and scaling. The patients were randomly subdivided into two groups. No preoperative antimicrobial therapy was administered. The third molars were removed by the same oral surgeon (UT). The flap was repositioned and sutured (Ethicon, 3-0 silk 632H, Johnson&Johnson, Belgium). All patients received post-operative instructions. Group 1st, the test group, used a % 0.2 chlorhexidine glu-

TABLE I

The percentage and scores of contaminated length of suture materials

No	Group 1 (CHX)	Group 1 (CHX)	Group 2 (Saline)	Group 2 (Saline)
1	47.5 %	2	77.5 %	4
2	46.9 %	2	89.1 %	4
3	75.4 %	3	86.3 %	4
4	73.1 %	3	89 %	4
5	84.1 %	4	79.1 %	4
6	75.3%	3	84.1 %	4
7	79 %	4	84.2 %	4
Mean±SD	68.7±15.1	3±0.8	84.2±4.5	4±0.0
Significance [a]	P<0.01	P<0.05	P<0.01	P<0.05

[a] *The Mann-Whitney U test*

conate post-surgical mouth rinse; Group 2nd, the control group, used saline. Patients were given antibiotics (amoxicillin, 2 g per day for 5 days) and analgesic drugs (naproxen sodium, 550 mg per day for 3 days). The sutures were removed after 7 days and kept in 70% alcohol. The investigator performing suture removal was unaware of which type of mouth rinses had been used.

For SEM processing, the material was critical-point dried (Balzers, Liechtenstein) using the dry ice method[20], sputter-coated with gold in a Polaron E5000 (Polaron, Watford, UK) and were observed in a field emission SEM (JSM-5600, JEOL Ltd., Tokyo, Japan). Digital SEM micrographs were taken along the whole length of every suture at 200x. This provided a complete picture of the debris (length per cent of debris contamination) was measured. The observer was blind to the groups. For statistical analysis, contamination of the suture surface was scored as follows: 0=no contamination; 1= 1-25% of suture length contaminated with debris; 2= 26-50% of suture length contaminated; 3= 51-75% of suture length contaminated; 4= 76-100% of suture length contaminated.

The Mann-Whitney U test was used to compare the contamination of silk suture materials between the 1st and the 2nd groups.

RESULTS

SEM observations showed that all of the silk sutures both test and control groups were encrusted with plaque attachment on the surface and in the interfilamental spaces (Fig. 1, 2). The surface of silk sutures in the 2nd group was completely contaminated by debris along the surface (Fig. 3). There were obvious differences in the amount of plaque and debris accumulated between the 1st and the 2nd groups (Fig. 4, 5, 6, 7). The Mann-Whitney U test (Table 1) showed significant differences in contamination between the 1st and the 2nd groups (P<0.01). The amount of contamination was significantly lower in the 1st group.

In the 2nd group, higher magnification of SEM showed numerous microorganisms which were predominantly rod-shaped bacteria (Fig. 8, 9) and higher magnification showed this debris to be composed mainly of clusters of spherical submicron organisms with some filamentous structures (Fig. 10, 11).

DISCUSSION

The approach clearly revealed the expected result that chlorhexidine was significantly effective on the amount of plaque and debris accumulation on silk sutures according to the evaluation of

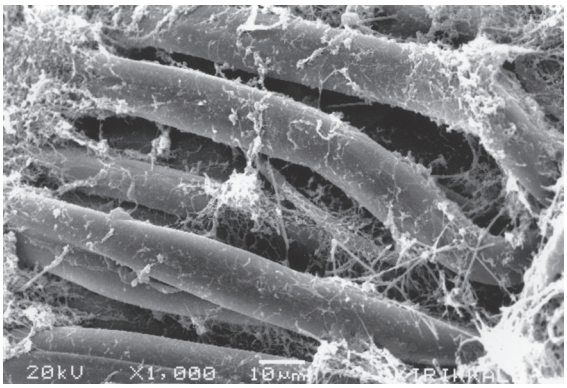

FIGURE 1

Scanning electron micrograph of silk suture of the test group. Plaque accumulation is seen on the surface and in the interfilamental spaces (1000X).

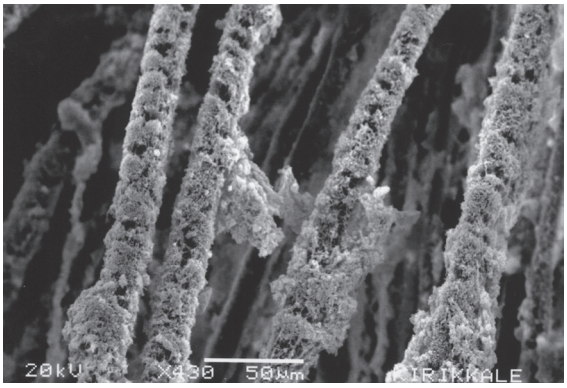

FIGURE 2

Scanning electron micrograph of silk suture of the control group. Plaque accumulation is seen in the interfilamental spaces (430X).

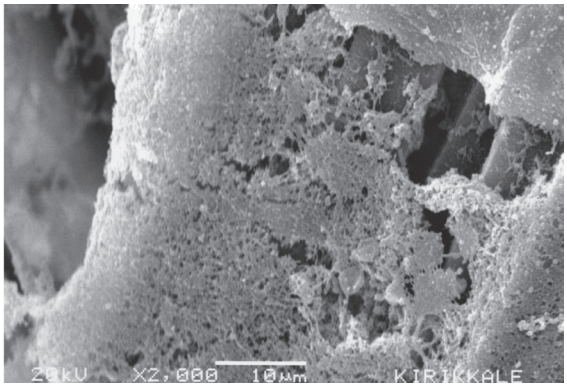

FIGURE 3

Scanning electron micrograph of silk suture of the control group. A large amount of debris is seen on the surface (2000X).

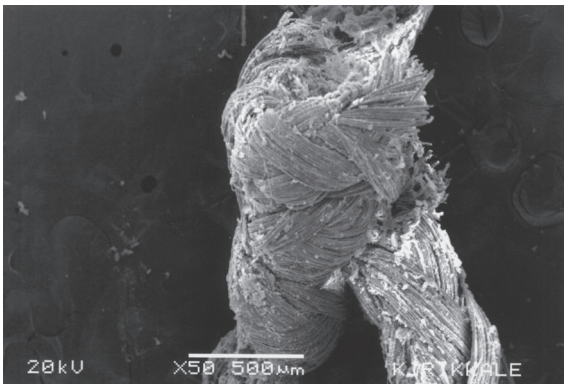

FIGURE 4

Scanning electron micrograph of silk suture of the test group showed some debris (50X).

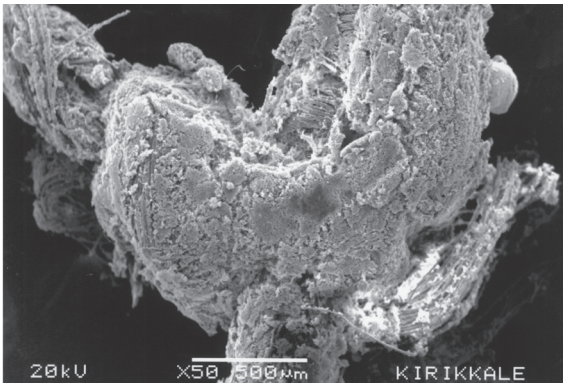

FIGURE 5

Scanning electron micrograph of silk suture of the control group showed a large amount of debris (50X).

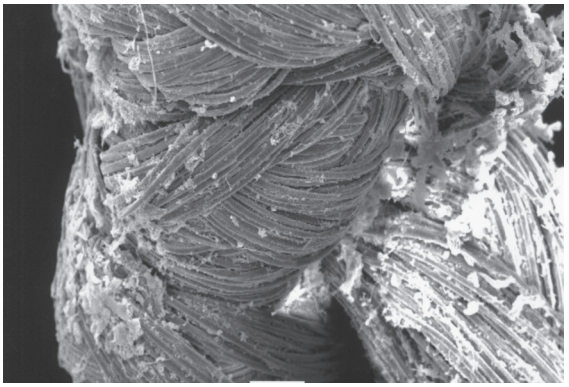

FIGURE 6

Scanning electron micrograph of silk suture of the test group showed some debris (120X).

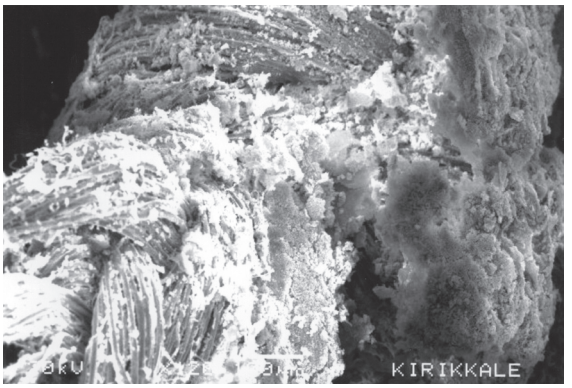

FIGURE 7

Scanning electron micrograph of silk suture of the control group showed a large amount of debris (120X).

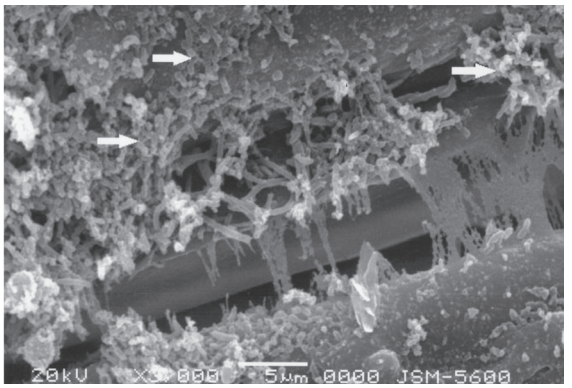

FIGURE 8

Scanning electron micrograph with higher magnification (3000X) of silk suture of the control group. A colony of rods could be seen.

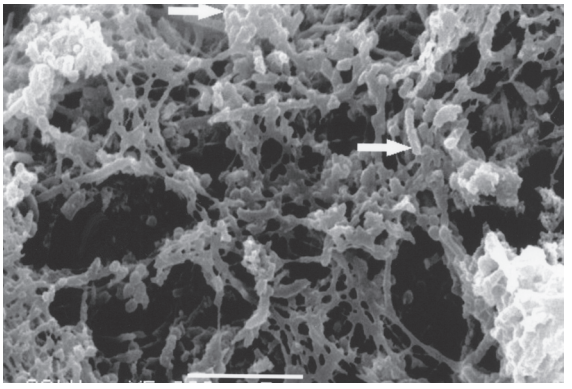

FIGURE 9

Scanning electron micrograph with higher magnification (5000X) of silk suture of the control group. A colony of rods could be seen.

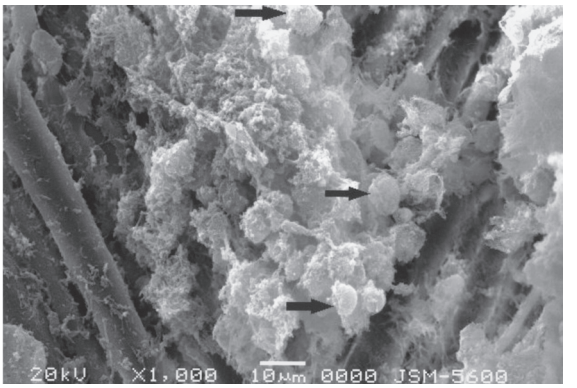

FIGURE 10

Scanning electron micrograph with higher magnification (1000X) of silk suture of the control group. A colony of spherical submicron organisms could be seen.

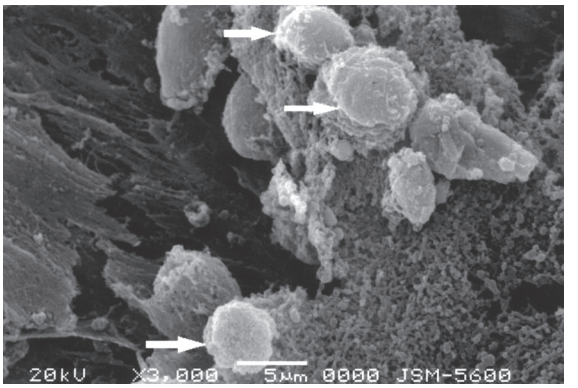

FIGURE 11

Scanning electron micrograph with higher magnification (3000X) of silk suture of the control group. A colony of spherical submicron organisms could be seen.

scanning electron micrographs and to our knowledge this is the first study that shows clearly the effect of CHX on silk sutures by SEM.

For comparison of the cleaning effect by rinsing, saline was used for the control group and a chlorhexidine product was used for the test group. All solutions were supplied by the manufacturer. Due to the double blind design of this study, the researchers who evaluate the contamination were unaware of the groups. Because of very different appearance of the silk and other suture materials such as polyvinylidene fluoride (PVDF), it is impossible for the observer to be

blind to the suture types. Therefore single type suture material was used in this study.

Bacterial plaque accumulation on the surface of sutures has been infrequently studied. Lilly et al[21] and Racey et al[22] suggested that greater inflammatory reactions in the oral mucosa have been produced by silk sutures than monofilament sutures. Parirokh et al[23] examined plaque accumulation on silk and PVDF sutures at different time intervals. They showed that PVDF sutures were contaminated less than silk sutures at 3, 5 and 7 days in SEM observation. In literature there is only one study that evaluates the plaque accumulation on suture materials by SEM[23]. Therefore, our data cannot be compared directly with many other studies. The evaluation of contamination of sutures was made according to the study of Parirokh et al[23] in our study. In accordance with, our results showed similar findings with this study, that all of the silk sutures both test and control groups were encrusted with plaque attachment on the surface and in the interfilamental spaces. But there were significant differences in contamination between the 1st and the 2nd groups.

Selvig et al[15] observed the greater inflammatory reaction with multi-filament materials and suggested that braided sutures seem to conduct bacterial migration to a great extent than monofilament sutures. In the same study the researchers reported that at 14 days bacterial plaque extended more than 1 mm into the suture channel regardless of the suture materials. In another study, silk sutures at 3 days showed a thick layer of bacterial plaque and debris[23]. In our study, the results were evaluated at only one time interval that is 7 days due to sutures are generally removed in this period.

The use of the SEM for viewing the surface of specimens is ubiquitous across all scientific disciplines and it offers a useful method to identify the amount of plaque accumulation on the materials of interest. SEM observation showed that the test group had less contaminated surface area than the control group. The antibacterial mode of action of CHX is explained by the fact that the cationic chlorhexidine molecule is rapidly attracted by the negatively charged bacterial cell surface. After adsorption, the integrity of the bacterial cell membrane is altered, which results in a reversible leakage of bacterial low molecular-weight components at low dosage[24] or more severe membrane damage at higher doses[25,26]. Moreover, CHX has the advantage of prolonged supragingival substantivity because it can bind to the intraoral soft and hard tissues[27]. Although there are many studies that evaluates the antiplaque effect of CHX in literature[8,28,29], our study clearly reveals the effectiveness of CHX on the amount of plaque accumulation on silk sutures by using SEM.

REFERENCES

1. Mandel ID. Chemotherapeutic agents for controlling plaque and gingivitis. J Clin Periodontol. 1988; 15: 488–498.
2. Brecx M. Strategies and agents in supragingival chemical plaque control. Periodontol 2000 1997; 15: 100–108.
3. Hickel R. Wirkstoffe gegen Plaque und Bakterien. Quintessenz Bonusausgabe. 1997; 1:45–57.
4. Jones CG. Chlorhexidine: is it still the gold standard? Periodontol 2000. 1997; 15:55-62.
5. Gjermo P. Chlorhexidine in dental practice. J Clin Periodontol. 1974; 1(3):143-52.
6. Addy M. Chlorhexidine compared with other locally delivered antimicrobials. A short review. J Clin Periodontol. 1986; 13(10):957-64.
7. Addy M, Greenman J, Renton-Harper P, Newcombe R, Doherty F. Studies on stannous fluoride toothpaste and gel (2). Effects on salivary bacterial counts and plaque regrowth in vivo. J Clin Periodontol. 1997; 24(2):86-91.
8. Rosin M, Welk A, Kocher T, Majic-Todt A, Kramer A, Pitten FA. The effect of a polyhexamethylene biguanide mouthrinse compared to an essential oil rinse and a chlorhexidine rinse on bacterial counts and 4-day plaque re-growth. J Clin Periodontol. 2002; 29: 392-399.
9. Sreenivasan P, Gaffar A. Antiplaque biocides and bacterial resistance: a review. J Clin Periodontol. 2002; 29: 965-974.
10. Guggenheim B, Regolati B, Schmid R & Mühlemann HR. Effects of the topical application of mutanase on rat caries. Caries Res. 1980; 14: 128–135.

11. Lindquist B, Edward S, Torell P, Craspe B. Effect of different caries preventive measures in children highly infected with mutans streptococci. Scand J Dent Res. 1989; 97: 330-337.
12. Gendron R, Grenier D, Sorsa T, Mayrand D. Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine. Clin Diagn Lab Immunol. 1999; 6:437–439.
13. Rölla G & Melsen B. On the mechanism of plaque inhibition by chlorhexidine. J Dent Res (spec issue B). 1975; 54:57–62.
14. Jenkins S, Addy M & Wade W. The mechanism of action of chlorhexidine. A study of plaque growth on enamel inserts in vivo. J Clin Periodontol. 1988; 15: 415–424.
15. Selvig KA, Biagiotti GR, Leknes KN, Wikesjö UME. Oral tissue reactions to suture materials. IJPRD. 1998; 18: 475-487.
16. Kim S. Endodontic microsurgery. In: Cohen S, Burns RE. Pathways of the pulp 8th ed. St Louis, MO, USA Mosby, 2002: 475.
17. Morrow SG, Rubinstein RA. Endodontic surgery. In: Ingle JI, Backland L. Endodontics. 5th ed. Ontario, Canada: B.C Decker; 2002: 709.
18. Wallace WR, Maxwell GR, Cavalaris CJ. Comparison of polyglycolic acid suture to black silk, chromic and plain catgut in human oral tissues. Oral Surg Oral Med Oral Pathol. 1970; 28:739-46.
19. Okamoto T, Gabrielli MF, Gabrielli MA. Influence of different types of non-resorbable suture material on the healing of extraction wounds--a histological study in rats. J Nihon Univ Sch Dent. 1990; 32(2):104-15.
20. Tanaka K, Iino A. Critical point drying method using dry ice. Stain Technology. 1974; 49:203-206.
21. Lilly GE, Osbon DB, Hutchinson RA, Heflich RH. Clinical and bacteriological aspects of polyglicolic acid sutures. J Oral Surg. 1973; 31:103-5.
22. Racey GL, Wallace WR, Cavalaris CJ, Marguard JV. Comparison of a polyglicolic-polylactic acid suture to black silk and plain catgut in human oral tissues. Oral Surg Oral Med Oral Pathol. 1978; 36:766-70.
23. Parirokh M, Asgary M, Eghbal MJ, Stowe S, Kakoei S. A scanning electron microscope study of plaque accumulation on silk and PVDF suture materials in oral mucosa. Int Endod J. 2004; 37:776-781.
24. Denton GW. Chlorhexidine. In: Block SS. Disinfection, sterilization and preservation. 4th ed. Philadelphia: Lea and Febiger, 1991; 274–289.
25. Kuyyakanond T, Quesnel LB. The mechanism of action of chlorhexidine. FEMS Microbiol Lett. 1992; 100:211–215.
26. Rölla G, Melsen B. On the mechanism of plaque inhibition by chlorhexidine. J Dent Res (spec issue B). 1975; 54:57–62.
27. Bonesvoll P, Gjermo P. A comparision between chlorhexidine and some quaternary ammonium compounds with regard to retention, salivary concentration and plaque-inhibiting effect in the human mouth after mouth rinses. Arch Oral Biol. 1978; 23:289–294.
28. Quirynen M, Avontroodt P, Peeters W, Pauwels M, Coucke W, Van Steenberghe D. Effect of different chlorhexidine formulations in mouthrinses on de novo plaque formation. J Clin Periodontol. 2001; 28:1127-1136.
29. Yates R, Shearer BH, Huntington E, Addy M. A method to compare four mouthrinses. J Clin Periodontol. 2002; 29:519-523.

CORRESPONDING ADDRESS

Ebru OLGUN ERDEMİR DDS, PhD

Kırıkkale University Faculty of Dentistry Department of Periodontology 71200 Kırıkkale TURKEY

Phone: +90 312 224 49 27 Fax : +90 312 225 06 85 e-mail: olgun_ebru@yahoo.com