Original Article

Antimicrobial Effectiveness of a Highly Concentrated Chlorhexidine Varnish Treatment in Teenagers with Fixed Orthodontic Appliances

Rengin Attin[a]; Anika Ilse[b]; Carola Werner[c]; Annette Wiegand[a]; Thomas Attin[d,e]

ABSTRACT

Objective: To evaluate the recolonization pattern of Mutans streptococci (*ms*) on densely colonized teeth with fixed orthodontic appliances after treatment with a highly concentrated (36%) chlorhexidine varnish.

Materials and Methods: Healthy subjects (n = 19) with fixed orthodontic appliances and high bacterial *ms* counts in saliva were recruited. In order to establish a baseline registration, plaque adjacent to brackets was sampled and cultivated on Dentocult® strips. Following professional tooth cleaning, chlorhexidine varnish was applied on all teeth for 8 minutes. The degree of recolonization with *ms* was assessed 2 weeks after varnish application in plaque around the brackets. For statistical analysis, the data were subjected to a repeated measures design.

Results: After 2 weeks, *ms* counts were reduced as compared to baseline values. However, the reduction only weakly met statistical significance ($P = .049$).

Conclusions: The application of a highly concentrated chlorhexidine varnish in patients with fixed orthodontic appliances does not result in a distinct reduction of *ms* numbers 2 weeks after treatment.

KEY WORDS: Orthodontic treatment; Caries prevention; Antibacterial varnish; Chlorhexidine; *Streptococcus mutans*

INTRODUCTION

Caries-preventive measures—good oral hygiene, establishment of noncariogenic dietary habits, and regular fluoride supplementation—often are not sufficient to prevent the occurrence of new carious lesions in orthodontic patients with high caries activity. Beyond a certain caries activity level, neither an increase in the frequency of tooth-brushing nor an increase in the dosage of administered fluoride is suitable to effectively stop the demineralization process in high-risk individuals.[1,2] Patients undergoing orthodontic therapy are subjected to oral ecologic changes that lead to increased numbers of *Streptococcus mutans* in saliva and plaque.[3,4]

It has also been shown that orthodontic treatment with fixed appliances results in enamel demineralization and an increased number of carious lesions, predominantly in sites adjacent to brackets.[5] Therefore, preventive efforts in these risk group have concentrated on direct suppression of the cariogenic microflora by chemotherapeutics as an adjunct to improved oral hygiene.

Chlorhexidine is the most potent documented antimicrobial agent against Mutans streptococci (*ms*) and dental caries. Different modes of administration are recommended for caries prevention.[6–8] It has been suggested that chlorhexidine application in the form of a varnish results in longer-lasting suppression of *ms* concentrations by chlorhexidine compared with other forms of application.[9–11] High and low concentrations have been reported to reduce the number of *ms* in plaque and saliva for considerable periods of time.[12–14]

[a] Associate Professor, Department of Operative Dentistry and Preventive Dentistry and Periodontology, University of Göttingen, Göttingen, Germany.

[b] Research Fellow, Department of Operative Dentistry and Preventive Dentistry and Periodontology, University of Göttingen, Göttingen, Germany.

[c] Associate Professor, Department of Medical Statistics, University of Göttingen, Göttingen, Germany.

[d] Professor, Department of Operative Dentistry and Preventive Dentistry and Periodontology, University of Göttingen, Göttingen, Germany.

[e] Department of Preventive Dentistry, Periodontology and Cariology, University of Zürich, Zürich, Switzerland.

Corresponding author: Dr. Rengin Attin, University of Göttingen, Department of Operative Dentistry, Robert-Koch Str 40, Göttingen, Germany (e-mail: renginattin@yahoo.de)

Accepted: November 2005. Submitted: July 2005.

 DOI: 10.2319/070605-218

Numerous studies have examined highly concentrated chlorhexidine varnishes as supersaturated solutions of chlorhexidine diacetate in ethanol, stabilized by the natural resin sandarac.[15–19] In these examinations, the optimal chlorhexidine varnish concentration suggested for suppression of *ms* amounted to 36% chlorhexidine, as represented by the varnish EC40® (Dentres, Nijmegen, The Netherlands).

The consensus of these studies was that *ms* were significantly suppressed for at least 4 weeks after a single chlorhexidine varnish application. This effect has been tested on teeth free of orthodontic appliances. On the other hand, studies performed in high-risk orthodontic patients with highly concentrated varnish treatment did not show any influence on the caries increment.[20] A rapid recolonization of the teeth with *ms* and a return to baseline values was regarded as a possible reason for the failure of chlorhexidine treatment in orthodontic patients.

Orthodontic patients usually are seen at appointments every 4–6 weeks. Therefore, it is desirable that suppression of *ms* caused by antimicrobial treatment last for at least this period of time. Hence it is of interest to evaluate whether suppression of *ms* within a suitable, and for orthodontic patients common, recall interval is possible. Moreover, as yet it is not known whether highly concentrated chlorhexidine varnishes are effective in suppressing recolonization of *ms* when applied in patients with fixed orthodontic appliances.

Therefore, the objective of the present study was to investigate the time period in which *ms* in patients with densely colonized teeth and fixed orthodontic appliances return to baseline values after a single treatment with the 36% chlorhexidine varnish EC 40®.

MATERIALS AND METHODS

Participants

The participants and their guardians gave informed consent for taking part in the study. Thirty-two volunteers with fixed orthodontic appliances treated in a private practice were screened, and 19 of them (median age, 14 years) were selected. The appliances had been inserted at least 2 months prior to the start of the study. All study participants fulfilled the inclusion criteria of high levels of *ms* in saliva as demonstrated by at least a score of 2 identified with the chair-side Strip-mutans® method according to Jensen and Bratthall.[21] None of the subjects had detectable frank caries lesions or defective restorations. Moreover, clinical examination and radiographs did not reveal any lesions on interproximal tooth surfaces.

Study Design

Professional tooth cleaning was performed and oral hygiene instructions were given to the participants prior to the study. Before baseline examination, the subjects refrained from all oral hygiene measures for 24 h. At baseline, the *ms* levels in plaque and saliva were recorded.

The plaque *ms* scores were determined with the site-specific modified Strip-mutans® technique (Orion Diagnostica, Espoo, Finland) as originally described by Wallman and Krasse[22] and modified by Twetman.[31] The number of colony-forming units (CFU) with characteristic morphology was screened and scored 0–3. The evaluation was blinded. Scores were as follows:

- 0 indicates no CFU (*ms* below detection level).
- 1 indicates 1–10 CFU, corresponding to approximately $< 10^4$–10^5 CFU
- 2 indicates 10–100 CFU, corresponding to approximately 10^5–10^6 CFU
- 3 indicates $>$100 CFU, corresponding to $>10^6$ CFU.

Selected teeth for plaque sampling were isolated with cotton rolls and dried. A small sterile brush was carefully brushed on the sites around the brackets of eight teeth (teeth 11, 14, 22, 25, 31, 34, 42, and 45) in each of the 19 patients. This means that a total of 152 sites were evaluated.

All brackets had been placed by the same orthodontist with etching gel, bonding material, and light-curing composite (Transbond XT®, 3M Unitec®, Neuss, Germany). In case of an extracted premolar, the adjacent premolar was evaluated. Sampled plaque was immediately spread on the roughened side of the plastic strip from the Strip-mutans® kit (Orion Diagnostica, Espoo, Finland). The strips were allowed to dry for 5 minutes at room temperature and were then incubated for 48 hours in a liquid medium. The composition of the medium was similar to the composition of mitis salivarius agar, with a sucrose concentration increased to 30%. Addition of a bacitracin disc from the kit results in a final concentration of 0.36 U of bacitracin per ml of medium.[23] Additionally, a saliva Strip-mutans® test was performed for each participant and evaluated. After 48 hours cultivation in the liquid medium, the scores of *ms* in plaque were recorded with the aid of a stereomicroscope with 10–25$\times$ magnification.[24]

The 19 subjects fulfilling the inclusion criteria were treated with EC40® at the next recall 1 week later. EC40® is a highly concentrated chlorhexidine varnish as supersaturated solution of chlorhexidine diacetate in ethanol, stabilized by the natural resin sandarac.[17,25–28] The varnish is available in glass ampoules containing approximately 1.5 mL varnish. The ampoules fit into the normal syringe used for anesthesia. For application of varnish, a wide needle with an inner diameter of 0.8 mm. was used

Prior to each varnish application, the orthodontic

arch wire was removed and the teeth were professionally cleaned with a rubber cup and pumice paste. The interdental areas were cleaned with unwaxed dental floss. Each quadrant was isolated with cotton rolls and dried with compressed air. EC40® was applied to all teeth with a brush and delivered into the interproximal areas with unwaxed dental floss. Following the manufacturer's advice, the varnish was left in place for 8 minutes and then removed with a brush. Two weeks after varnish treatment, *ms* levels in plaque were recorded on the buccal sites as described above.

Statistical Methods

All microbiological measurements were recorded on a grading scale; the observations are so-called ordered categorical data, and thus standard statistical methods such as the *t*-test or analysis of variance cannot be applied. For the analysis of such data, ranking methods have been developed.[29] Therefore, the original observations are replaced with their ranks. Because only the four grades 0, 1, 2, and 3 are possible values, many observations will have the same values, which are called ties in the statistical literature. When ranking tied observations are given, midranks are assigned. Because ranking methods are used for the analysis of the data, it is reasonable to use the relative treatment effects to summarize the outcome of the trial in tables and graphs.

The statistical design underlying the observations in our trial is a repeated measures design, ie, the same patients are repeatedly observed at several time points. This means that statistically significant differences are assumed when time profiles are not parallel to the x-axis. For a detailed description of these methods, see Brunner et al.[30]

RESULTS

The statistical analysis was done as described above using relative treatment effects. For better illustration of the original data, the distributions of *ms* score changes during the 2-week interval are given in a cross-table (Table 1). The table demonstrates that a deterioration to higher scores occurred in 36 cases, whereas in 64 sites an improvement to lower scores took place. Scores for the other 52 sites remained unchanged. However, *ms* scores were below detection level (score 0) in only 34 sites at the end of the 2-week period.

In Figure 1, the results of site-specific plaque samples on teeth with brackets at baseline (T0) and 2 weeks (T1) after varnish treatment are demonstrated. After 2 weeks, *ms* counts were reduced as compared to baseline values. The reduction did meet statistical significance, but only weakly ($P = 0.049$). In the design of the study, we intended to record *ms* levels in plaque every 2 weeks until *ms* values returned to baseline values. Because in nearly every subject recolonization with *ms* was already complete after 2 weeks of varnish application, recording of *ms* values was not continued.

Nevertheless, we could observe intra-individual differences. The degree of reinfection varied considerably between different patients (Table 2). However, only 2 patients showed a distinct suppression of *ms* counts.

TABLE 1. Cross-tabulated Number of *ms* Values Indicating Toothwise Changes From Baseline to Two Weeks[a]

	ms Score	2 wk 0	1	2	3	Total
Baseline	0	**4**	6	5	0	15
	1	4	**6**	7	1	18
	2	11	8	**17**	17	53
	3	15	6	20	**25**	66
	Total	34	26	49	43	152

[a] Numbers set in **bold** are *ms* scores that remained unchanged during the two experimental weeks. Entries above bold numbers demonstrate numbers of sites with deterioration in *ms* scores; entries below bold numbers illustrate numbers of sites with improved *ms* scores. *ms* indicates Mutans streptococci.

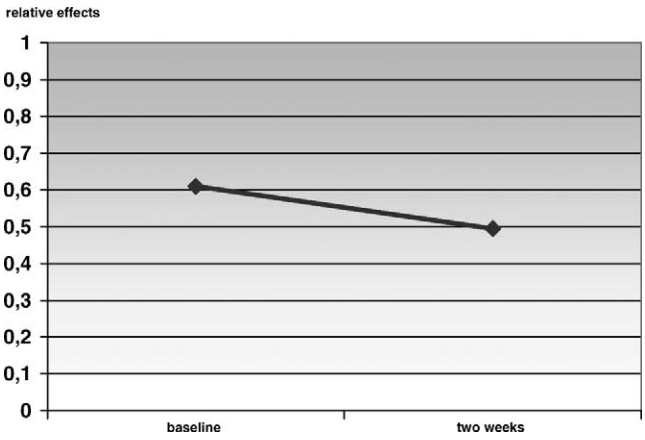

Figure 1. Relative effects of *ms* counts in plaque at baseline and 2 weeks after treatment with EC40®.

DISCUSSION

The *ms* counts in the present study were evaluated with a commercial available *S. mutans* test, namely the chair-side Strip-mutans-Dentocult® test.[31] The reliability of this method has been proven by numerous studies. There is a significant correlation between conventional analysis with MSB agar[32] and the Strip-mutans® test.[33,34] Intra-individual differences with the Strip-mutans® method were investigated by El-Nadeef

TABLE 2. Exemplary Plaque *ms* Scores (0–3) in a 13-Year-Old Girl and a 14-Year-Old Boy at Baseline and 2 Weeks After Treatment Given for the Respective Teeth at Which Plaque Samples Were Taken[a]

	13-Year-Old Girl		14-Year-Old Boy	
Tooth	Baseline	2 wk	Baseline	2 wk
14	3	1	3	3
11	3	1	2	3
22	3	2	3	3
25	2	1	3	3
34	3	2	2	3
31	2	1	1	2
42	1	1	1	2
45	1	0	3	2

[a] *ms* indicates Mutans streptococci.

and Bratthall.[35] They observed that tests that were repeated in one subject usually showed no differences. Tests varied in more than one category in very rare cases. Therefore, with good handling, the Strip-mutans® method is a very reliable method.

If fluoridation measures and dietary counseling are not considered, the use of antimicrobials is currently the only promising alternative to improved oral hygiene. However, it must be noted that studies performed in high-risk orthodontic patients did not find significant differences in caries increment after repeated application of high- or low-concentration chlorhexidine varnishes.[36,37] In contrast to these findings, other investigations with orthodontic patients have documented a reducing effect of chlorhexidine and/or fluorides on caries increment and *S. mutans*.[38–42] However, in contrast to the present investigation, in these studies patients taking part in the trials were not preselected with regard to caries risk, caries activity or levels of *ms* in plaque and saliva, respectively,

In a former study it was proved that the efficacy of a highly concentrated varnish is reduced by bands and brackets.[43] In this split-mouth–designed study, recolonization on teeth with orthodontic appliances occurred significantly faster than on teeth without appliances.

It was assumed that the duration of *ms* suppression depends partly on the extent to which any retention niches are coated with varnish. In a previous study by Jenatschke et al,[44] *ms* counts were assessed only at baseline and 8 weeks, so the definite time point of the recolonization was not discernible. In that study, the varnish was applied on the day of bracket placement and was repeated at 8-week intervals, while the fixed appliances were in place. As mentioned above, in this investigation the caries increment was not reduced despite the use of chlorhexidine varnish treatment. It can be assumed that the recolonization must have taken place during this time interval (ie, 8 weeks) so that recolonization with *ms* and caries development could not be avoided.

The recolonization with *ms* after antimicrobial therapy in highly colonized teeth was investigated in the present study. We could show that *ms* counts had nearly returned to baseline values after 2 weeks. Because only a weak significant difference between baseline and 2-week values was observed, the reduction of *ms* is assumed to be not clinically relevant. This suggestion is corroborated by the fact that most of the samples were not below detection level 2 weeks after varnish treatment.

Additionally, it could be assumed that a complete recolonization would have taken place within a short period after completion of the 2-week interval chosen in the present study. This finding may act as an explanation of why no effect on caries increment was achieved in former studies. On the other hand, an application modus applying the varnish even more often, eg, once a week, is not practicable. A more effective application mode must be found to hamper the fast recolonization of *ms* in orthodontic patients.

Emilson and Lindquist[45] evaluated the coherence of the infection level of *ms* and recolonization of teeth after chlorhexidine treatment and observed that tooth surfaces with a high level of infection are more rapidly colonized by *ms.* This was true even if these microorganisms had previously been suppressed to undetectable levels after antimicrobial treatment. This observation makes clear that a slow recolonization pattern and higher efficacy more presumably can be achieved in teeth with low levels of colonization.

Unfortunately, patients with a fast recolonization pattern are more likely to develop carious lesions after application of fixed orthodontic appliances. Therefore, an effective way of suppressing *ms* should be found for these patients. An effective way could be to apply the varnish immediately before bracketing and banding of the teeth. It may be hypothesized that this results in a slower recolonization of *ms.* Afterwards, the exact recolonization time after varnishing has to be determined to set an application interval and to suppress *ms* during the entire therapy with fixed appliances. Another focus should be set on the cleaning of the tongue, because the tongue may act as an infection source, possibly favoring the recolonization pattern on the teeth. Of course, it can also be assumed that in the present study no suppression at all could be achieved. This is speculative, because no *ms* counts were assessed right after varnish treatment. The authors had expected suppression for at least 4 weeks, and because of this assumption, the *ms* counts were investigated after a 2-week interval.

Therefore, development of more effective therapy modes and identification of reasons for lack of effec-

tiveness need to be investigated in further studies. Furthermore, interdisciplinary work between the cariologist and orthodontist should be intensified to develop caries prevention strategies for orthodontic patients, because these patients are at a high caries risk.[46]

CONCLUSIONS

- A single 36% chlorhexidine varnish application is not effective in suppression of *ms* counts to a clinically relevant degree in patients with fixed orthodontic appliances and highly colonized *ms* salivary counts.

REFERENCES

1. Øgaard B, Larsson E, Glans R, Henriksson T, Birkhed D. Antimicrobial effect of a chlorhexidine-thymol varnish (Cervitec) in orthodontic patients. A prospective, randomized clinical trial. *J Orofac Orthop.* 1997;58:206–213.
2. Øgaard B, Seppä L, Rølla G. Professional topical fluoride applications—clinical efficacy and mechanism of action. *Adv Dent Res.* 1994;8:190–201.
3. Lundström F, Krasse B. Caries incidence in orthodontic patients with high levels of *Streptococcus mutans*. *Eur J Orthod.* 1987;9:117–121.
4. Lundström F, Krasse B. *Streptococcus mutans* and lactobacilli frequency in orthodontic patients; the effect of chlorhexidine treatments. *Eur J Orthod.* 1987;9:109–116.
5. Mitchell L. Decalcification during orthodontic treatment with fixed appliances—an overview. *Br J Orthod.* 1992;19:199–205.
6. Emilson CG. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. *J Dent Res.* 1994;73:682–691.
7. Fardal O, Turnbull RS. A review of the literature on use of chlorhexidine in dentistry. *J Am Dent Assoc.* 1986;112:863–869.
8. Zickert I, Emilson CG, Krasse B. Effect of caries preventive measures in children highly infected with the bacterium *Streptococcus mutans*. *Arch Oral Biol.* 1982;27:861–868.
9. Attin R, Tuna A, Attin T, Brunner E, Noack MJ. Efficacy of differently concentrated chlorhexidine varnishes in decreasing Mutans streptococci and lactobacilli counts. *Arch Oral Biol.* 2003;48:503–509.
10. Emilson CG. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. *J Dent Res.* 1994;73:682–691.
11. Pienihäkkinen K, Söderling E, Ostela I, Leskela I, Tenovuo J. Comparison of the efficacy of 40% chlorhexidine varnish and 1% chlorhexidine-fluoride gel in decreasing the level of salivary mutans streptococci. *Caries Res.* 1995;29:62–67.
12. Sandham HJ, Brown J, Chan KH, Phillips HI, Burgess RC, Stokl AJ. Clinical trial in adults of an antimicrobial varnish for reducing mutans streptococci. *J Dent Res.* 1991;70:1401–1408.
13. Sandham HJ, Brown J, Phillips HI, Chan KH. A preliminary report of long-term elimination of detectable mutans streptococci in man. *J Dent Res.* 1988;67:9–14.
14. Schaeken MJ, De Haan P. Effects of sustained-release chlorhexidine acetate on the human dental plaque flora. *J Dent Res.* 1989;68:119–123.
15. Keltjens HM, Schaeken MJ, van der Hoeven JS, Hendriks JC. Effects of chlorhexidine gel on periodontal health of abutment teeth in patients with overdentures. *Clin Oral Implants Res.* 1991;2:71–74.
16. Schaeken MJ, Keltjens HM, van der Hoeven JS. Effects of fluoride and chlorhexidine on the microflora of dental root surfaces and progression of root-surface caries. *J Dent Res.* 1991;70:150–153.
17. Schaeken MJ, Schouten MJ, Van Den Kieboom CW, van der Hoeven JS. Influence of contact time and concentration of chlorhexidine varnish on mutans streptococci in interproximal dental plaque. *Caries Res.* 1991;25:292–295.
18. Schaeken MJ, De Haan P. Effects of sustained-release chlorhexidine acetate on the human dental plaque flora. *J Dent Res.* 1989;68:119–123.
19. Schaeken MJ, van der Hoeven JS, Hendriks JC. Effects of varnishes containing chlorhexidine on the human dental plaque flora. *J Dent Res.* 1989;68:1786–1789.
20. Jenatschke F, Elsenberger E, Welte HD, Schlagenhauf U. Influence of repeated chlorhexidine varnish applications on mutans streptococci counts and caries increment in patients treated with fixed orthodontic appliances. *J Orofac Orthop.* 2001;62:36–45.
21. Jensen B, Bratthall D. A new method for the estimation of mutans streptococci in human saliva. *J Dent Res.* 1989;68:468–471.
22. Wallman C, Krasse B. A simple method for monitoring mutans streptococci in margins of restorations. *J Dent.* 1993;21:216–219.
23. Jensen B, Bratthall D. A new method for the estimation of mutans streptococci in human saliva. *J Dent Res.* 1989;68:468–471.
24. Twetman S, Frostner N. Salivary mutans streptococci and caries prevalence in 8-year-old Swedish schoolchildren. *Swed Dent J.* 1991;15:145–151.
25. Keltjens HM, Schaeken MJ, van der Hoeven JS, Hendriks JC. Effects of chlorhexidine gel on periodontal health of abutment teeth in patients with overdentures. *Clin Oral Implants Res.* 1991;2:71–74.
26. Schaeken MJ, Keltjens HM, van der Hoeven JS. Effects of fluoride and chlorhexidine on the microflora of dental root surfaces and progression of root-surface caries. *J Dent Res.* 1991;70:150–153.
27. Schaeken MJ, De Haan P. Effects of sustained-release chlorhexidine acetate on the human dental plaque flora. *J Dent Res.* 1989;68:119–123.
28. Schaeken MJ, van der Hoeven JS, Hendriks JC. Effects of varnishes containing chlorhexidine on the human dental plaque flora. *J Dent Res.* 1989;68:1786–1789.
29. Brunner E, Langer F. Nonparametric analysis of ordered categorical data in designs with longitudinal observations and small sample sizes. *Biometric J.* 2000;42:663–675.
30. Brunner E, Domhof S, Langer F. Nonparametric analysis of longitudinal data in factorial designs. New York, NY: Wiley; 2002.
31. Twetman S. Eine einfache Methode zur Überprüfung der Wirkung der topikalen Behandlung mit einem antibakteriellen Lack. *Zahnärztl Welt.* 1995;104:38–40.
32. Gold OG, Jordan HV, Van Houte J. A selective medium for *Streptococcus mutans*. *Arch Oral Biol.* 1973;18:1357–1364.
33. Jensen B, Bratthall D. A new method for the estimation of mutans streptococci in human saliva. *J Dent Res.* 1989;68:468–471.
34. Kneist S, Heinrich-Weltzien R, Stößer L. A comparison between commercial kits and conventional methods for the enumeration of mutans streptococci. *Caries Res.* 1996;30:267–312.
35. El-Nadeef M, Bratthall D. Intraindividual variations in counts

of mutans streptococci measured by ''Strip mutans'' method. *Scand J Dent Res.* 1992;100:149–153.
36. Jenatschke F, Elsenberger E, Welte HD, Schlagenhauf U. Influence of repeated chlorhexidine varnish applications on mutans streptococci counts and caries increment in patients treated with fixed orthodontic appliances. *J Orofac Orthop.* 2001;62:36–45.
37. Lundström F, Krasse B. Caries incidence in orthodontic patients with high levels of *Streptococcus mutans*. *Eur J Orthod.* 1987;9:117–121.
38. Madlena M, Vitalyos G, Marton S, Nagy G. Effect of chlorhexidine varnish on bacterial levels in plaque and saliva during orthodontic treatment. *J Clin Dent.* 2000;11:42–46.
39. Denes J, Gabris K. Results of a 3-year oral hygiene programme, including amine fluoride products, in patients treated with fixed orthodontic appliances. *Eur J Orthod.* 1991; 13:129–133.
40. Dyer JR, Shannon IL. MFP versus stannous fluoride mouthrinses for prevention of decalcification in orthodontic patients. *ASDC J Dent Child.* 1982;49:19–21.
41. Eronat C, Alpoz AR. Effect of Cervitec varnish on the salivary Streptococcus mutans levels in the patients with fixed orthodontic appliances. *J Marmara Univ Dent Fac.* 1997;2: 605–608.
42. Geiger AM, Gorelick L, Gwinnett AJ, Benson BJ. Reducing white spot lesions in orthodontic populations with fluoride rinsing. *Am J Orthod Dentofacial Orthop.* 1992;101:403–407.
43. Attin R, Thon C, Schlagenhauf U, Werner C, Wiegand A, Hannig C, Attin T. Recolonization of mutans streptococci on teeth with orthodontic appliances after antimicrobial therapy. *Eur J Orthod.* 2005;27:489–493.
44. Jenatschke F, Elsenberger E, Welte HD, Schlagenhauf U. Influence of repeated chlorhexidine varnish applications on mutans streptococci counts and caries increment in patients treated with fixed orthodontic appliances. *J Orofac Orthop.* 2001;62:36–45.
45. Emilson CG, Lindquist B. Importance of infection level of mutans streptococci for recolonization of teeth after chlorhexidine treatment. *Oral Microbiol Immunol.* 1988;3:64–67.
46. Mitchell L. Decalcification during orthodontic treatment with fixed appliances—an overview. *Br J Orthod.* 1992;19:199–205.