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DOI: 10.2478/aoj-2013-0008

Effect of chlorhexidine varnish application on *Streptococcus mutans* colonisation in adolescents with fixed orthodontic appliances

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Objective: The aim of this prospective study was to evaluate the re-colonisation pattern of *Streptococcus mutans* (MS) in high-level MS-colonised patients with fixed orthodontic appliances following 40% chlorhexidine varnish application prior to bracket placement.

Materials and methods: The subjects of this single-blinded clinical trial were 13-14-year-old adolescents (N = 14) with significant orthodontic treatment need, a high salivary MS count but without any carious lesions. Baseline MS levels were determined by the cultivation of saliva collected from each subject using strips developed for this purpose (Strip-mutans, Orion Diagnostica, Espoo, Finland). Prior to the bonding of orthodontic brackets, 40% chlorhexidine varnish (EC 40, Explore, Nijmegen, Netherlands) was applied to all teeth for 10 minutes. The re-colonisation of MS was assessed at one, two, four and six week time periods. The data obtained were subjected to a repeated measures design.

Results: Chlorhexidine varnish reduced salivary MS significantly at the first, second and fourth weeks compared to baseline values. Significant MS suppression lasted less than six weeks and MS colonisation gradually returned to baseline level.

Conclusion: Repeated application of chlorhexidine varnish in orthodontic patients with high MS levels may be beneficial throughout fixed appliance orthodontic treatment.

(Aust Orthod J 2013; 29: 52-57)

Received for publication: June 2012
Accepted: February 2013

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Introduction

Dental caries is a consequence of the production and continuous challenge of organic acids by cariogenic bacteria, which eventually results in enamel demineralisation.[1] Preventive measures involving good oral hygiene, a non-cariogenic diet and regular fluoride supplements, are often not sufficient to prevent this undesired process in orthodontic patients who express high caries activity.[1] Beyond a certain level, neither an increase in the frequency of tooth brushing nor an increase in the dosage of administered fluoride are effective in stopping the demineralisation process in high-risk individuals.[2] The oral microbiota in orthodontic patients with fixed appliances shows increased numbers of *Streptococcus mutans* (MS) in saliva which remains the most powerful factor in caries prediction models.[2-5]

An increased incidence of enamel demineralisation and carious lesions has been reported in patients undergoing orthodontic treatment.[6,7] Caries risk increases with the insertion of orthodontic devices, as fixed appliances create retentive areas which are

difficult to mechanically clean and which lead to the accumulation of plaque and cariogenic micro-organisms.[6,7] Therefore, preventive efforts in these risk groups mainly target the direct suppression of the micro-organisms by chemotherapeutics and the remineralisation of early decalcification.[8-10]

Chlorhexidine (CHX), used in the prevention of dental caries, is one of the most effective antimicrobial agents against MS.[11] Different modes of administration have been recommended which involve mouth rinses and varnishes.[12-14] It has been demonstrated that CHX varnish application results in a longer-lasting suppression of MS compared with other forms of application.[14-16] Schaeken et al. achieved the inhibition of MS in plaque and saliva for considerable periods of time using varying concentrations of CHX in varnishes.[17-19] High concentration CHX varnishes, as supersaturated solutions of chlorhexidine-di-acetate in ethanol stabilised by natural resin sandarac were found to be most effective at a suggested CHX concentration of 40 per cent.[19-21]

Schaeken et al. showed that MS was significantly suppressed for at least four weeks after a single CHX varnish application in appliance-free patients.[19-21] However, Jenatschke et al. reported that the use of highly concentrated CHX varnish had no influence on the caries involvement of patients wearing orthodontic appliances.[22] Even though the CHX varnish application was repeated every eight weeks until the end of treatment, long-term bacterial suppression was not achieved and the plaque retentive areas were claimed to be responsible for this unfavourable result.[22] Attin et al.[23] further confirmed that eight weeks after CHX varnish application, the MS population had gradually returned to its baseline value. Therefore, knowledge of the exact re-population time of MS in highly colonised orthodontic patients after antimicrobial therapy would appear valuable, in order to determine a varnish re-application scheme to achieve long-term bacterial suppression.

The objective of the present study was to investigate the effect and re-colonisation pattern of 40% CHX varnish on MS levels of high-level MS-colonised orthodontic patients undergoing fixed appliance treatment over a six-week period. The null hypothesis to be tested was that 40% CHX varnish applied before bracket placement has no long-term suppressive effect on MS in dental plaque of high-level MS-colonised patients undergoing fixed appliance treatment.

Materials and methods

Subjects

Subjects identified to participate in the study provided informed, written consent. Forty patients treated in a private practice with the need for full fixed orthodontic appliances were screened and 14 (6 boys and 8 girls, mean age of 13 years), were selected. All fulfilled the inclusion criteria requiring high levels of MS in saliva (involving at least a score of 2 identified by a chair-side Strip-mutans® test method according to Jensen and Bratthall[24]), no detectable frank carious lesions or defective restorations and no detectable lesions on interproximal tooth surfaces. Prior to bracket placement, the patients were provided with routine oral hygiene instruction requiring toothbrushing twice per day with a fluoridated toothpaste.

Saliva sampling

To facilitate saliva sampling, subjects were instructed to refrain from all oral hygiene measures for 24 hours. The samplings were performed in the mornings and the participants were required not to eat or drink for at least 1 hour before the sampling. Samples of paraffin-stimulated saliva were tested by means of special strips (Dentocult® SM Strip-mutans, Orion Diagnostica, Espoo, Finland) developed for this purpose, at baseline and again after one, two, four and six weeks.

Determination of Streptococcus mutans in saliva

The levels of salivary MS were estimated using the Dentocult® SM Strip-mutans according to the manufacturer's instructions. The strips were rotated on the back of the tongue and cultivated on selective agar for 48 hours at 37°C in semi-anaerobic conditions. The number of colony-forming units per square centimetre was estimated using a stereomicroscope (x10-25 magnification, Zeiss, Göttingen, Germany).

Evaluation of MS samples

The number of colony-forming units per ml saliva (CFU / ml saliva) were screened using a blinded approach and scored 0-3.[24-26]

A score of 0 represented no CFU / ml saliva (MS below detection level),

a score of 1 = 1-10 CFU / ml saliva, corresponding to $< 10^4$-10^5 CFU / ml saliva,

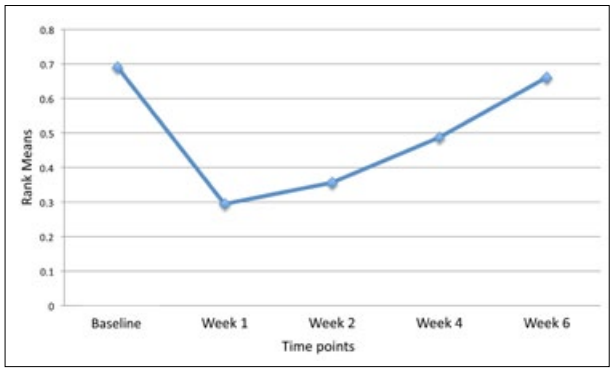

Figure 1. Rank means of *Streptococcus mutans* counts in saliva at baseline (TO) and 1 (T1), 2 (T2), 4 (T3) and 6 weeks (T4) after treatment with EC40®, respectively.

a score of 2 = 10-100 CFU / ml saliva, corresponding to 10^5-10^6 CFU / ml saliva, and

a score of 3 > 100 CFU / ml saliva, corresponding to >10^6 CFU / ml saliva.

MS levels of baseline, one, two, four and six week measurements after varnish treatment were similarly recorded.

Chlorhexidine varnish application

In preparation for varnish application, accessible tooth surfaces were cleaned with a rubber cup and fluoride-free pumice. The interdental areas were cleaned with unwaxed dental floss. Each quadrant was isolated with cotton rolls and dried with air. The teeth of all subjects were painted, with the provided brush, with 40% CHX varnish (EC40®, Explore, Nijmegen, Netherlands) while varnish delivery into interproximal areas was gained via unwaxed dental floss. The varnish was left in place for 10 minutes and removed with a brush and floss. Following varnish application, banding and bracketing were immediately performed.

Data analysis

The obtained categorical data formed by repeated measures scored using the grading scale were analysed using ranking methods[25-27] which replaced the original four grading scores with ranks. The smallest score in the data set was assigned a rank of 1, the second smallest score a rank of 2, and so on until all scores had been converted to ranks. Tied scores were assigned midranks (i.e. if the second and third score in the data set were the same number, the assigned ranks would be 2 and 3, but they would be given an average rank, or midrank, of (2 + 3) / 2 = 2.5). The assigned group ranks were summed and divided by the number of observations to calculate each group's mean rank. The time profiles of the rank means were tested at the 5% significance level ($p \leq 0.05$). Post-hoc analyses were performed by appropriate adjustment of the significance level in case of rejection of parallel time profiles.[27] More detailed description of the ranking method has been described elsewhere.[27,28]

Results

There was a statistically significant decrease in MS counts at the first ($p < 0.001$), second ($p < 0.001$) and fourth week ($p < 0.05$) measurements compared with the baseline level. The sixth week counts did not reveal a significant decrease ($p = 0.924$).

The number of patients who scored higher MS counts was the lowest at the first week time point and gradually increasing MS counts were observed at each time point thereafter. A return to baseline levels was reached at the end of the six-week interval. The percentage distributions of the bacterial counts are shown in Table I. Assigned rank means of MS in saliva and the change in time are shown in Figure 1.

Discussion

The present study was designed to gain information on the effect of 40% CHX varnish on the MS level and re-colonisation pattern of highly MS-colonised orthodontic patients undergoing fixed appliance treatment over a six-week period. The results demonstrated that the CHX varnish application did not prevent MS re-colonisation to baseline levels after six weeks and repeated applications might be beneficial in order to support other caries preventive measures. The null hypothesis was accepted.

The MS counts in the present study were evaluated with a commercially-available chair-side Strip-mutans® test. The reliability of this method has been previously proven and identified through a significant correlation between a conventional analysis with mitis salivarius bacitracin agar and the Strip-mutans®.[24,29] El-Nadeef and Bratthall investigated the individual differences with the Strip-mutans method[30] and observed that repeated tests performed on one subject showed no significant discrepancy. Only in rare cases, high intra-examiner variances were

Table I. Number of subjects with average saliva scores (0-3) for MS (*Streptococcus mutans*) at baseline (T0) and 1 (T1), 2 (T2), 4 (T3), 6 (T4) weeks after varnish treatment. Percentages are given in parentheses.

MS score	T0	T1	T2	T3	T4
3	8 (57%)	1 (7%)	4 (29%)	4 (29%)	4 (29%)
2	6 (43%)	3 (21%)	1 (7%)	8 (57%)	9 (64%)
1	0 (0%)	4 (26%)	7 (50%)	2 (14%)	0 (0%)
0	0 (0%)	6 (43%)	2 (14%)	0 (0%)	1 (7%)
∑	14 (100%)	14 (100%)	14 (100%)	14 (100%)	14 (100%)

observed. Therefore the Strip-mutans® method was considered reliable with its expedient handling characteristics.

The anti-bacterial effect of the CHX varnish may be explained by the radical damage to the cell membrane of prokaryotes and the disruption of cytoplasmic contents which results in cell death.[11] The elimination of almost all MS in a single application of high concentration CHX varnish within the limits of the painted areas has been reported previously.[15,16] This reduction effect is also assumed to result in a delayed bacterial re-colonisation compared with treatments using low concentration agents.[23] However, the disadvantage of CHX application is its transient effect and the regrowth of MS over time.[31] The presence of protected sites beyond the range of professional tooth cleaning continues to harbour MS so that complete elimination is impossible.[16] Due to the high viscosity of the varnish and its inability to flow into retentive areas, MS could not be totally eliminated. It is therefore reasonable to assume that the remaining MS bacteria were likely responsible for the re-colonisation of the teeth. The suppressant effect of the varnish could possibly be ameliorated by using low-abrasive air-polishing methods incorporating glycine or sodium bicarbonate containing powders to clean the teeth and to reduce the level of MS prior to varnish application. An alternative possibility might be additional cleaning of the tongue as its rough surface harbours micro-organisms, which also promote the dental re-colonisation pattern.

Although many studies provide evidence of the caries-preventive effect of CHX derivatives, the present study was inconclusive in regard to children and adolescents without fixed orthodontic attachments.[31-34] However, the need for further studies to test the effective concentrations of CHX, sufficient to control MS, was highlighted.[32] Accordingly, studies that particularly targeted caries prevention with CHX in orthodontic patients were carried out by several investigators.[4,22,33] The results failed to demonstrate a significant decrease in caries incidence after repeated applications using high or low concentration CHX varnishes in orthodontic patients with a high caries risk.[4,22,33] However, these studies applied antibacterial therapy when appliances were already in place.

To address the concern and potential effect of fixed attachments, Attin et al. tested the efficacy of a highly concentrated varnish and showed that the caries-preventive effect reduced because of attached orthodontic bands and brackets.[23] In a split-mouth design study, re-colonisation of teeth with fixed attachments occurred significantly faster than on teeth with smooth surfaces. It was considered that the duration of MS suppression partly depended on the extent to which any retention niches were coated with varnish. Due to the time intervals evaluated, at baseline and after eight weeks, the definite time point of the re-colonisation was unable to be determined.[23] Beyth et al. confirmed that salivary MS levels increased after bracket placement and decreased following CHX application.[34]

In a recent study, Attin et al. showed that no long-lasting suppression of MS could be achieved in patients with highly colonised teeth undergoing fixed appliance therapy.[28] CHX was applied when the appliances were in place and, two weeks after CHX treatment, no significant MS reduction was observed. In the present study, highly concentrated CHX varnish was applied prior to appliance placement in order to increase the time before MS re-colonisation. This strategy may have lead to a significantly decreased MS count four weeks after varnish application compared with baseline levels. This is contrary to previously reported results in which a return to baseline values was observed over this period.

As orthodontic patients are usually recalled every four to six weeks, it would be beneficial if the antimicrobial suppression of MS lasted for at least this period of time. The present study demonstrated that MS re-colonisation time could be prolonged so that a significant reduction was still present after four weeks. This result suggested a prevention strategy in which patients could be screened for high caries risk before orthodontic treatment and highly concentrated varnish applied immediately before bracketing. During the course of orthodontic therapy, varnish treatments would be repeated at four-weekly-intervals. Further studies employing this strategy are needed to confirm the effect on caries susceptibility.

Conclusion

The repeated application of chlorhexidine varnish in orthodontic patients with high MS levels may be beneficial in four to six weekly follow-up intervals in order to support other caries preventive measures. The development of more effective applications providing long-term suppression of MS should be the aim of further studies.

Acknowledgment

The authors gratefully acknowledge Professor Edgar Brunner, University of Göttingen, Germany, for his statistical advice.

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References

1. Øgaard B, Rølla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1. Lesion Development. Am J Orthod Dentofacial Orthop 1988;94:68-73.
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;9:117-21.
4. Lundström F, Krasse B. Streptococcus mutans and lactobacilli frequency in orthodontic patients: the effect of chlorhexidine treatments. Eur J Orthod 1987;9:109-16.
5. Petersson GH, Isberg PE, Twetman S. Caries risk assessment in school children using a reduced Cariogram model without saliva tests. BMC Oral Health 2010;10:5.
6. Boersma JG, van der Veen MH, Lagerweij MD, Bokhout B, Prahl-Andersen B. Caries prevalence measured with QLF after treatment with fixed orthodontic appliances: influencing factors. Caries Res 2005;39:41-7.
7. Richter AE, Arruda AO, Peters MC, Sohn W. Incidence of caries lesions among patients treated with comprehensive orthodontics. Am J Orthod Dentofacial Orthop 2011;139:657-64.
8. Øgaard B, Rølla G, Arends J, ten Cate JM. Orthodontic appliances and enamel demineralization Part 2. Prevention and treatment of lesions. Am J Orthod Dentofacial Orthop 1988;94:123-8.
9. Behnan SM, Arruda AO, Gonzales-Cabezas C, Sohn W, Peters MC. In-vitro evaluation of various treatments to prevent demineralization next to orthodontic brackets. Am J Orthod Dentofacial Orthop 2010;138:712.e1-712.e7.
10. Kronenberg O, Lussi A, Ruf S. Preventive effect of ozone on the development of white spot lesions during multibracket appliance therapy. Angle Orthod 2009;79:65-9.
11. Scheie AA. The role of antimicrobials. In: Fejerskov O, Kidd E, eds. Dental caries: The disease and its clinical management. Copenhagen: Blackwell Publishing, 2003;179-88.
12. Zickert I, Lindvall AM, Axelsson P. Effect on caries and gingivitis of a preventive program based on oral hygiene measures and fluoride application. Community Dent Oral Epidemiol 1982;10:289-95.
13. Fardal O, Turnbull RS. A review of the literature on use of chlorhexidine in dentistry. J Am Dent Assoc 1986;112:863-9.
14. Emilson CG. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. J Dent Res 1994;73:682-91.
15. 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;29:62-7.
16. 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;48:503-9.
17. 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.
18. 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;70:150-3.
19. Schaeken MJ, van der Hoeven JS, Hendriks JC. Effects of varnishes containing chlorhexidine on the human dental plaque flora. J Dent Res 1989; 68:1786-9.
20. Schaeken MJ, De Haan P. Effects of sustained-release chlorhexidine acetate on the human dental plaque flora. J Dent Res 1989;68:119-23.
21. 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;25:292-5.
22. 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.
23. Attin R, Thon C, Schlagenhauf U, Werner C, Wiegand A, Hannig C et al. Recolonization of mutans steptococci on teeth with orthodontic appliances after antimicrobial therapy. Eur J Orthod 2005;27:489-93.

24. Jensen B, Bratthall D. A new method for the estimation of mutans streptococci in human saliva. J Dent Res 1989;68:468-71.
25. Wallman C, Krasse B. A simple method for monitoring mutans streptococci in margins of restorations. J Dent 1993;21:216-19.
26. Twetman S, Frostner N. Salivary mutans streptococci and caries prevalence in 8-year-old Swedish schoolchildren. Swed Dent J 1991;15:145-51.
27. Brunner E, Domhof S, Langer F. Nonparametric analysis of longitudinal data in factorial designs. New York: Wiley; 2002. Chapter 8.3:130-52.
28. Attin R, Ilse A, Werner C, Wiegand A, Attin T. Antimicrobial effectiveness of a highly concentrated chlorhexidine varnish treatment in teenagers with fixed orthodontic appliances. Angle Orthod 2006;76:1022-7.
29. Gold OG, Jordan HV, Van Houte J. A selective medium for Streptococcus mutans. Arch Oral Biol 1973;18:1357-64.
30. El-Nadeef MA, Bratthall D. Intraindividual variations in counts of mutans streptococci measured by "Strip mutans" method. Scand J Dent Res 1991;99:8-12.
31. Twetman S. Antimicrobials in future caries control? A review with special reference to chlorhexidine treatment. Caries Res 2004;38:223-9.
32. James P, Parnell C, Whelton H. The caries-preventive effect of chlorhexidine varnish in children and adolescents: a systematic review. Caries Res 2010;44:333-40.
33. Derks A, Frencken J, Bronkhorst E, Kuijpers-Jagtman AM, Katsaros C. Effect of chlorhexidine varnish application on mutans streptococci counts in orthodontic patients. Am J Orthod Dentofacial Orthop 2008;133:435-9.
34. Beyth N, Redlich M, Harari D, Friedman M, Steinberg D. Effect of sustained-release chlorhexidine varnish on Streptococcus mutans and Actinomyces viscosus in orthodontic patients. Am J Orthod Dentofacial Orthop 2003;123:345-8.