Clin Oral Invest (2010) 14:107–112
DOI 10.1007/s00784-009-0273-5

ORIGINAL ARTICLE

Effects of mouthrinses containing essential oils and alcohol-free chlorhexidine on human plaque acidogenicity

Katarina Wikén Albertsson · Anitha Persson · Peter Lingström · Jan W. V. van Dijken

Received: 26 August 2008 / Accepted: 24 March 2009 / Published online: 29 April 2009
© Springer-Verlag 2009

Abstract The aim of this in vivo study was to evaluate the effect of two antimicrobial mouthrinses on dental plaque acidogenicity after a sucrose challenge. Twenty subjects, with a mean age of 59 years, participated in a double-blind intraindividual randomized study. Three mouthrinses were used in 16-day rinsing periods in addition to their regular mechanical oral hygiene: a solution with essential oils (EO), solution with alcohol-free chlorhexidine (CHX) and water (negative control). The three test periods were separated by 3-month washout periods. Changes in plaque acidogenicity were evaluated after a sucrose challenge at day 0 (baseline) and at day 17 of each mouthrinse period using the microtouch method. Both CHX and EO resulted at day 17 in statistically significant less attenuated pH falls compared to the water rinse. The CHX mouthrinse resulted in the least pronounced pH values compared with EO (ns) during the whole 30-min period. When calculated as area under the curve (AUC), significantly lower values ($AUC_{6.2}$) were found for CHX and EO at day 17 compared to day 0. A significant difference for $AUC_{6.2}$ between CHX and water was found at day 17. No statistically significant differences were found for any of the comparisons with $AUC_{5.7}$. The results from this study indicate that both the essential oils and the alcohol-free chlorhexidine reduced plaque acidogenicity after a sucrose challenge. Large interindividual variations were observed.

Keywords Acids · Chlorhexidine · Dental plaque · Essential oils · Mouthrinse · Plaque pH

K. Wikén Albertsson · A. Persson · J. W. V. van Dijken
Department of Odontology, Dental Hygienist Education,
Faculty of Medicine, Umeå University,
Umeå, Sweden

P. Lingström
Department of Cariology,
Institute of Odontology at Sahlgrenska Academy,
University of Gothenburg,
Gothenburg, Sweden

K. Wikén Albertsson (✉)
Department of Odontology, Dental Hygienist Education,
Umeå University,
SE-901 87 Umeå, Sweden
e-mail: katarina.albertsson@odont.umu.se

Introduction

Dental plaque forms naturally on the teeth. In the absence of adequate oral hygiene, it can accumulate beyond levels that are compatible with dental caries, which may then develop at susceptible sites. Caries prevalence has declined during the last decades not at least by increased customary tooth cleaning with fluoridated toothpaste [16]. Even if the largest effect has been attributed to the frequent fluoride exposure, also the cleaning effect is stated to be of importance. In several individuals, the level of oral hygiene is still insufficient at many sites in order to provide a good plaque control consistent with oral health. During the last years, there has been a reemerged interest in the use of mouthrinses for chemical plaque control as adjunct to daily mechanical plaque control.

Chlorhexidine, a bis-biguanide, is currently the most potent chemotherapeutic agent against the cariogenic microflora [14]. Natural susceptibility varies with more potent effects on Gram-positive than on Gram-negative bacteria. Among cariogenic microorganisms, the mutans streptococci are particularly sensitive, while lactobacilli are less susceptible [3]. Chlorhexidine has often been used as a positive control during assessment of the anticariogenic potential of other agents [3, 15]. However, the side effects of chlorhexidine, primarily staining and taste alteration, limit its potential to be used for long-term use.

A solution containing four different essential oils (thymol, eucalyptol, menthol, and methyl salicylate) in a 22% hydroalcoholic vehicle has also been found to have a negative effect on oral bacteria [15]. At high concentrations, disrupting of cell walls of bacteria and precipitation of cell proteins has been reported and at lower concentrations inactivation of essential enzymes [15]. The solution prevents oral bacteria from aggregating with Gram-positive pioneer species and slows down bacterial multiplication [11]. Stoeken et al. [15] reviewed recently the literature on the effectiveness of this essential oil-containing mouthrinse against plaque and gingivitis. Four studies showed that it was significantly more effective in reducing plaque and gingivitis compared to a 5% hydroalcohol control. However, Charles et al. [2] found no difference in effect on plaque and gingivitis between chlorhexidine and essential oils (2004), while other studies have shown that chlorhexidine was more effective [6, 12]. Despite the long history of this mouthwash with essential oils, its effect on caries and cariogenicity of the dental plaque has hardly been studied. Fine et al. [4] reported a significant reduction in proportion of *S. mutans* to total streptococci in interproximal plaque, but not in saliva, sampled 1 h after a mouthrinse with essential oils in a 12-day experimental period. Up to today, no study has evaluated the effect of essential oils on plaque pH fall after exposure to fermentable carbohydrates in vivo.

The hypothesis tested in this study was that the use of a mouthrinse containing essential oils was as effective as alcohol-free chlorhexidine in order to prevent plaque acidogenicity. The aim of this study was to evaluate the effect of daily use of mouthrinses containing essential oils with respective alcohol-free chlorhexidine, in addition to regular mechanical oral hygiene, on changes in plaque pH after a sucrose challenge.

Material and methods

Test subjects

Twenty healthy volunteers (12 men and eight women) with a mean age of 58.9 years (range 42–90 years), recruited at the Dental school, University of Umeå, Sweden, participated in the study. The subjects showed a mean DMFS of 61.2, a mean stimulated whole salivary flow rate of 2.0 ml/min, and a mean saliva buffer capacity of 5.2 pH units (final pH). All, except two, subjects had a history of high caries frequency. Oral and written information was given to the subjects at the first visit. All subjects gave their informed consent prior to starting. The study was approved by the Regional Research Ethics Committee at the medical faculty, Umeå University (Dnr 08-072 M).

Study design

The study was designed as a double-blind randomized intraindividual comparison of three mouthrinses: (1) a solution containing essential oils (Listerine, McNeil, Stockholm, Sweden; EO), 20 ml, (2) a solution with 0.12 % alcohol-free chlorhexidine (Paroex; Sunstar Butler, Mölndal, Sweden; CHX), 10 ml, and (3) water (negative control), 10 ml. Each product was used during a test period lasting for 16 days in addition to their regular mechanical oral hygiene procedures. The subjects refrained from brushing during 3 days before each mouthrinse started and the last 3 days of each rinsing period. The different mouthrinse periods, which were carried out in randomized order, were separated by 3-month washout periods. The unsupervised rinsings were initiated after a baseline oral examination and plaque pH measurement (day 0). The amount and use of the products were used according to the manufacturer's recommendations. The subjects used the mouthrinses once on day 0 and twice a day during days 1–16. In order to assure compliance, they maintained a diary to document the rinses as well as the performance of the daily mechanical oral hygiene procedures.

Plaque pH measurements

Plaque pH measurements were performed at the dental clinic, School of Dentistry, University of Umeå at baseline (day 0) and at the end of the three mouthrinse periods (day 17) by use of the microtouch method [8]. Each subject attended six plaque pH measurement sessions. On days 0 and 17, the subjects were not allowed to eat, drink, or use tobacco for 2 h prior to the pH measurements. As a reference electrode, a plate of silver–silver chloride (ECG type Synectics Medical, Stockholm, Sweden) was placed on the skin of the forearm with an electrode gel (Spectra 360, Parker, Orange, NJ, USA) [13]. Measurements were carried out at two sites; in the right and left maxillary anterior region. All registration sites were free from metal restorations. A microelectrode (Beetrode® NMPH-1, WPI, Sarasota, FL, USA) was inserted into the interproximal plaque under the contact area. After measurement of the baseline plaque pH (0 min), the subjects rinsed for 1 min with 10 ml of a 10% sucrose solution (SIGMA, St Louis, MO, USA). Plaque pH was then measured at time points 2, 5, 10, 15, 20, and 30 min. The electrode was calibrated before each test session against two standard pH buffers at pH 7.00 and 4.01. Two operators experienced with the microtouch method performed the measurements; one handling the active electrode and the second as independent reader. The pH electrode was calibrated against standard buffer pH 7.00 between the measurements [13].

Statistical analysis

For each subject, mean plaque pH from the two measurement sites was calculated for each time point, after which the mean for each product was calculated. The data were processed in Statistical Package for the Social Sciences (SPSS, version 15.0, Chicago, IL, USA). For plaque-pH, individual pH curves were obtained for each of the mouthrinses at days 0 and 17. The pH curves were analyzed using the following variables: baseline pH (0 min) and final pH (30 min) values, minimum pH and maximum pH decrease during the 30-min measurement time period. For the critical pH levels of dentin (6.2) and enamel (5.7), the measured pH levels were estimated against time (pH×time). The total time period which plaque was below these critical levels was calculated as areas under the curve, $AUC_{5.7}$ and $AUC_{6.2}$, respectively. Differences between mean values of the studied pH variables were analyzed using a two factor analysis of variance (ANOVA) and Fischer's PLSD. A $p<0.05$ was considered statistically significant.

Results

There was a dropout of two subjects during the study, one participant did not attend all the sessions, and the other wanted to discontinue the mouthrinses. Thus, 18 subjects fulfilled the study.

No statistically significant differences were observed between the baseline pH measurements for the three rinsing periods. Changes in plaque pH days 0 and 17 for the three mouthrinses are shown as mean values for each test session in Fig. 1. The CHX mouthrinse resulted in the least pronounced pH falls during the whole 30-min period. No statistically significant differences were observed between the two active mouthrinse products. Both products resulted in significantly less pronounced pH falls compared to the water rinse within the first 20 min: EO vs water at 5 min ($p<0.05$) and CHX vs water at 2, 5, 10, and 20 min ($p<0.05$ or $p<0.01$).

Comparing plaque-pH at days 0 and 17, within each mouthrinse, showed for EO statistically significant differences between baseline and the first 10 min measurements ($p<0.05$ or $p<0.01$). For CHX, significant differences were observed between baseline and up to 20 min ($p<0.05$ or $p<0.01$). No difference was found between the different time points for the water rinse.

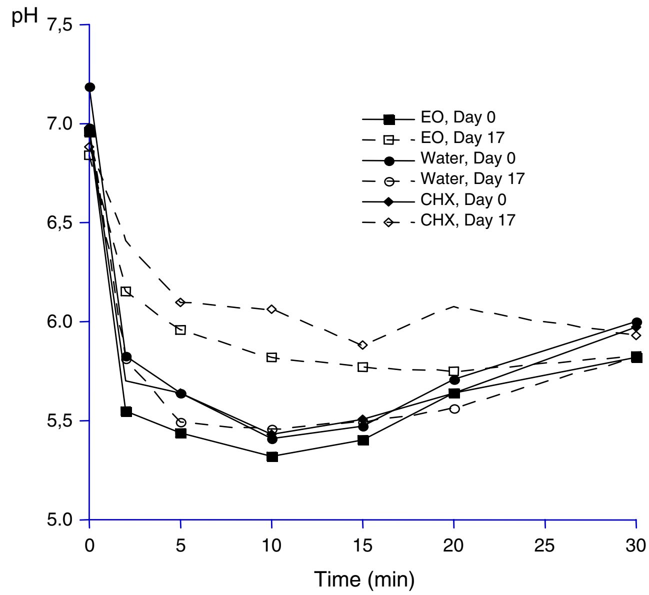

Fig. 1 Changes in plaque pH for the three rinsing periods using a solution with essential oils (EO), a solution with alcohol-free chlorhexidin (CHX) and water. The values at days 0 and 17 are given as mean values at each of the time points. $n=18$

Baseline pH, minimum pH, maximum pH decrease, and final pH for the mouthrinses are shown in Table 1. For minimum pH and maximum pH decrease, no significant differences were found between the mouthrinses at day 0. At day 17, CHX and EO showed significant differences with water for maximum pH decrease ($p<0.05$). For minimum pH, CHX was significantly different with water ($p<0.01$). No significant differences were observed between the active mouthrinsing products, EO and CHX, concerning baseline pH, minimum pH, maximum pH decrease, and final pH. Within the groups, a statistically significant difference was observed between days 0 and 17 for EO and CHX for both minimum pH ($p<0.001$) and maximum pH decrease ($p<0.01$).

Significantly lower $AUC_{6.2}$ was found for EO and CHX comparing the results from days 0 and 17 ($p<0.05$). A significant difference between CHX and water was found at day 17 ($p<0.05$). For the water control, no significant difference between measurements performed at days 0 and 17 was found ($p=0.652$; Fig. 2). A similar pattern for $AUC_{6.2}$ was seen for $AUC_{5.7}$, but no significant differences were found for any of the comparisons (data not shown). However, analyses of $AUC_{5.7}$ for EO between days 0 and 17 was close to significance ($p=0.072$).

Discussion

The solubility of enamel and dentin hydroxyapatite is greatly affected by the pH of dental plaque. Acid production in dental plaque after fermentation of ferment-

Table 1 Baseline pH, minimum pH, maximum pH decrease, and final pH at Day 0 and Day 17 for the three rinsing periods, using a solution with essential oils (EO), a solution with alcohol-free chlorhexidin (CHX) and water (n=18); mean±SD (min-max)

	Baseline pH	Minimum pH	Maximum pH decrease	Final pH
EO day0	6.96±0.54 (5.99–7.88)	5.10±0.52 (4.41–5.96)	1.97±0.80 (0.73–3.06)	5.82±0.68 (4.54–6.85)
EO day17	6.84±0.48 (5.93–7.87)	5.53±0.56 (4.36–6.43)	1.19±0.56 (0.41–2.75)	5.83±0.58 (4.64–6.44)
CHX day0	6.98±0.46 (6.01–7.66)	5.23±0.57 (4.23–6.20)	1.75±0.54 (0.84–2.56)	5.97±0.69 (4.31–7.16)
CHX day17	6.88±0.50 (6.13–7.71)	5.36±1.33 (4.34–6.55)	1.22±0.57 (0.47–2.24)	5.93±0.53 (4.61–7.01)
Water day0	7.19±0.50 (6.32–8.52)	5.24±0.49 (4.18–5.87)	1.91±0.40 (1.15–2.74)	6.00±0.51 (4.87–7.02)
Water day17	6.98±0.55 (5.97–7.96)	5.19±0.62 (3.70–6.23)	1.94±0.99 (0.76–3.06)	5.82±0.57 (4.71–6.91)

able carbohydrates by cariogenic bacteria, reflect the cariogenic challenge at the tooth surface. Different techniques have been described in order to assess changes of plaque-pH. Advantages of the microtouch method are its potential to follow the plaque pH changes after a fermentable carbohydrate challenge during a longer time period and the possibility to measure several sites at the same time point [8]. The large interindividual variations observed in the present study are confirmed by previous studies [13].

Substances that prevent or retard the acid production in plaque are important from a clinical perspective. The effect of chlorhexidine on plaque is well established [14]. An aqueous 0.05% fluoride–0.05% CHX diacetate solution on plaque acidogenicity has earlier been evaluated by Giertsen and Scheie [5]. They used the microtouch method after a 1-min sucrose mouthrinse and observed that the F-CHX-solution reduced the in vivo pH fall significantly compared with a F-solution only. This is in accordance with the findings for the 0.12% alcohol-free CHX solution compared to the water mouthrinse in the present study. Oppermann and Gjermo [10] compared the effect on in vivo plaque acidogenicity of different antimicrobial agents at different time intervals after previous sugar exposure using the touch method. Both 2% chlorhexidine and 70% ethanol inhibited pH drops for a 24-h period after treatment. Used in lower concentrations, a reduced effect was seen for ethanol, while chlorhexidine was capable of retaining its effect. The higher effectiveness of chlorhexidine was suggested to be related to its prolonged retention in the oral cavity after application.

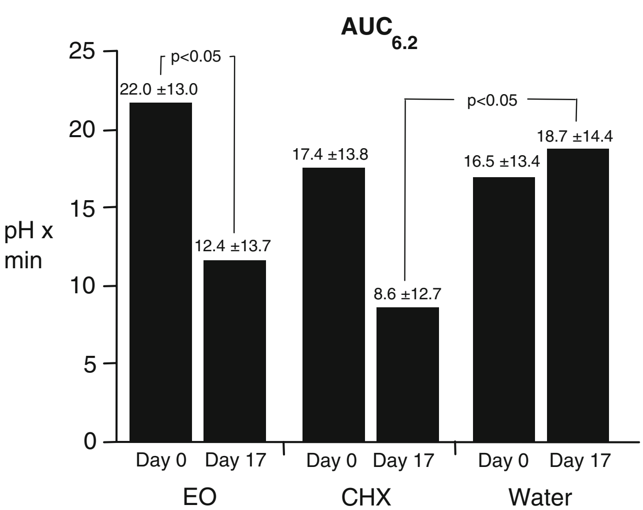

Fig. 2 The AUC $_{6.2}$ (pH x min) for the three rinsing periods using a solution with essential oils (EO), a solution with alcohol-free chlorhexidin (CHX) and water at day 0 and 17. n=18

Many commonly used commercial antimicrobial mouthrinses contain alcohol, an ingredient used for dissolution of active ingredients. One of the most established CHX solutions, Hibitane (ICI Dental), contains 0.07 ml 96% ethanol per milliliter, and Listerine contains a 0.225 ml 95% ethanol per milliliter. The role of alcohol in mouthrinses has been questioned, and it has been speculated that regular use of alcohol-containing mouthrinses can cause desiccation of the oral mucosal membranes and increase subjective xerostomia. Participants in the study by Oppermann and Gjermo [10] complained about soreness and a burning sensation of the gingival tissues after treatment with alcohol. This was one of the reasons why the alcohol-free chlorhexidine was tested in the present study. However, Kerr et al. [7] found no difference in the objective and subjective measures of dry mouth for subjects rinsing with an alcohol-containing compared to an alcohol-free mouthrinse. None of the participants in the present study complained about side effects of the CHX solution. One can speculate if the ethanol concentration of the commercial CHX solutions may have an additive effect on plaque-pH reduction, compared to the effect of 70% ethanol alone [10]. The effect of a non-alcohol-containing chlorhexidine treatment on plaque acidogenicity has not been investigated. Oppermann [9] studied the effect of 0.01–0.2% aqueous solutions of Hibitane on plaque acidogenicity and suggested that the lowest concentration to prevent a fall below pH 5.5, after a previous sucrose solution, was 0.05%. The CHX solution in the present study is one of the few commercial non-alcohol-containing antimicrobial mouthrinses. It showed the strongest effect on plaque-pH during the whole 30-min test period.

Mouthrinses containing essential oils have shown to reduce plaque accumulation. Their effect on extent and duration of pH fall after sucrose challenges have not been reported. Only one study evaluated its effect on plaque metabolic acids [18]. A 36% reduction in the amount of lactate, acetate, and proprionate in dental plaque was observed, which further was interpreted as a reduction in the total acidogenic activity of plaque microflora. The pH data from the present study confirm a reduced acid production after use of EO. Zhang et al [18] used a similar 14-day mouthrinse period, followed by 3 days abstaining from oral hygiene, with the last rinse only 60 min before sampling. A washout period of only 2 weeks was used between the EO and ethanol–water rinses. For CHX, it is known that 3-month washout periods are necessary, as used in the present study, but it is uncertain if the effect after EO rinses lasts longer than 2 weeks. The EO showed during the whole period of measurement a less pronounced effect on plaque-pH when compared to CHX, but the difference was not statistically significant. The hypothesis stated was therefore accepted. Both EO and CHX showed significantly lower pH changes compared with the control mouthrinse, i.e., water. The pH curves for CHX and EO showed that, after 17 days use of the mouthrinses, the mean plaque pH after a sucrose challenge was kept above the critical pH level of enamel during the whole 30-min measurement period, suggesting a caries preventive potential of both rinses against enamel caries. However, one has also to consider the considerably individual variance of critical pH levels in a population. To generalize the preventive effect, mentioned above, to all individuals is therefore not possible. Especially in subjects with higher critical pH values, individual pH decreases will cross the critical pH level, and demineralization will occur.

Many host factors influencing the caries disease, like plaque quality, salivary secretion rate and buffer capacity, intake frequency, and oral retention of food products, oral motorics, cariogenic microorganisms amount, and species are involved and affect the response of dental plaque to carbohydrates. Demineralization of tooth tissue depends on the cariogenic potential of foods in each individual's oral environment. The mean feature of the microtouch method is its capacity to provide evidence of the cariogenic potential of fermentable carbohydrates under normal conditions or as in the present study when participants completed their normal oral hygiene measures by a mouthrinse [8]. The intraindividual comparison model made it possible to compare the three mouthrinses within each of the participant potential cariogenic environments. The impact of both mouthrinses in the development of caries is uncertain. There are very few clinical studies of CHX mouthrinses, which assess caries progression and none with focus on EO mouthrinses. Recent reviews of CHX mouthrinses indicated its antimicrobial properties against cariogenic bacteria, buts its use as an anticaries agent is not clearly shown [1, 17]. The observed acidogenicity reduction in dental plaque may attribute to a caries preventive effect in parts of the subjects. The reduction can be attributed to several factors like reduction in plaque amount, changed plaque composition, plaque viability reduction, changes in plaque metabolism, or to shifts in the microflora. The results from this study indicate that both the essential oils and the alcohol-free chlorhexidine reduced plaque acidogenicity after a sucrose challenge. Large interindividual variations were observed.

Acknowledgements This study was supported by the Medical faculty of Umeå University. The mouthrinses were supplied by the manufacturers.

Conflict of interest The authors declare that they have no conflict of interest in the studied mouth rinsing products.

References

1. Autio-Gold J (2008) The role of chlorhexidine in caries prevention. Oper Dent 33:710–6
2. Charles CH, Mostler KM, Bartels LL, Mankodi SM (2004) Comparative antiplaque and antigingivitis effectiveness of a chlorhexidine and an essential oil mouthrinse: 6-month clinical trial. J Clin Periodontol 31:878–884
3. Emilson CG (1994) Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. J Dent Res 73:682–691
4. Fine DH, Furgang D, Barnett ML, Drew C, Steinberg L, Charles CH, Vincent JW (2000) Effect of an essential oil-containing antiseptic mouthrinse on plaque and salivary *Streptococcus mutans* levels. J Clin Periodontol 27:157–161
5. Giertsen E, Scheie AA (1995) Effects of mouthrinses with chlorhexidine and zinc ions combined with fluoride on the viability and glycolytic activity of dental plaque. Eur J Oral Sci 103:306–312
6. Grossman E, Meckel AH, Isaacs RL, Feretti GA, Sturzenberger OP, Bollmer BW, Moore DJ, Lijana RC, Manhart MD (1989) A clinical comparison of antibacterial mouthrinses: effects of chlorhexidine, phenolics, and sanguinarine on dental plaque and gingivitis. J Periodontol 60:435–440
7. Kerr AR, Katz RW, Ship JA (2007) A comparison of the effects of 2 commercially available nonprescription mouthrinses on salivary flow rates and xerostomia. Quintessence Int 38:440–447
8. Lingström P, Imfeldt T, Birkhed D (1993) Comparison of three different methods for measurement of plaque-pH in humans after consumptions of soft bread and potato chips. J Dent Res 72:65–70
9. Oppermann RV (1979) Effect of chlorhexidine on acidogenicity of dental plaque in vivo. J Dent Res 87:302–308
10. Oppermann RV, Gjermo P (1980) In vivo effect of four antibacterial agents upon the acidogenicity of dental plaque. Scand J Dent Res 88:34–39
11. Ouhayoun J-P (2003) Penetrating the plaque biofilm: impact of essential oil mouthwash. J Clin Periodontol 30(Suppl. 5):10–12
12. Overholser CD, Meiller TF, DePaola LG, Minah GE, Niehaus C (1990) Comparative effects of 2 chemotherapeutic mouthrinses on the development of supragingival dental plaque and gingivitis. J Clin Periodontol 17:575–579

13. Persson A, Lingström P, Bäcklund T, van Dijken JWV (2004) Evaluation of a skin reference electrode used for intraoral pH measurements in combination with a microtouch electrode. Clin Oral Investig 8:172–175
14. Ribeiro LGM, Hashizume LN, Maltz M (2007) The effect of different formulations of chlorhexidine in reducing levels of mutans streptococci in the oral cavity: a systematic review of the literature. J Dent 35:359–370
15. Stoeken JE, Paraskevas S, Godefridus A, van der Weijden GA (2007) The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: a systematic review. J Periodontol 78:1218–1228
16. Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, Lingström P, Mejare I, Nordenram G, Norlund A, Petersson LG, Söder B (2003) Caries-preventive effect of fluoride toothpaste: a systematic review. Acta Odontol Scand 61:347–355
17. Twetman S (2004) Antimicrobials in future caries control? A review with special reference to chlorhexidine treatment. Caries Res 38:223–229
18. Zhang JZ, Harper DS, Vogel GL, Schumacher G (2004) Effect of an essential oil mouthrinse, with and without fluoride, on plaque metabolic acid production and pH after a sucrose challenge. Caries Res 38:537–541

Copyright of Clinical Oral Investigations is the property of Springer Science & Business Media B.V. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.