The effects of three different mouth rinses in a 4-day supragingival plaque regrowth study

Feyza Ulkur¹, Tulin Arun², Fulya Ozdemir²

Correspondence: Dr. Feyza Ulkur
Email: feyza.ulkur@yeditepe.edu.tr

¹Department of Orthodontics, Faculty of Dentistry, Yeditepe University Istanbul, Turkiye
²Orthodontist, Private Practice, Istanbul, Turkiye

ABSTRACT

Objective: This study compares the effects of three different mouth rinses with respect to reducing Streptococcus mutans (S. mutans) colony counts on the teeth and tongue surfaces. Materials and Methods: In this study, comparison tests using the alcohol-free 0.1% chlorhexidine mouth rinse, alcohol-containing essential oil mouth rinse, and alcohol-free essential oil-containing mouth rinse were conducted. Patients were instructed to avoid mechanical cleaning with either a toothbrush or toothpick for 4 days. The first samples were collected from teeth surfaces and the dorsum of the tongue after a professional cleaning, and the second samples were collected after a 4-day plaque re-growth period. The supragingival plaque from the buccal surfaces of teeth #11, 14, 31, 34 as well as samples from the dorsum of the tongue, were assessed using the Dentocult® strips. Results: The Listerine® and Ondrohexidine® groups did not show any statistically significant differences between the values of the two samples (P = 0.734, P = 0.307). The MC® group and the control group showed significantly higher results than the first sample values. The effectiveness of the mouth rinses on S. mutans colony counts from the teeth surfaces were higher in the Listerine®, Ondrohexidine®, and Mouthwash Concentrate® groups. The difference between the first and second samples of the S. mutans colony counts from the tongue surface was found to be statistically significant, and S. mutans colony counts were higher than the first sample (P = 0.015). Conclusion: Alcohol and essential oil-containing Listerine® mouth rinse, alcohol-free Ondrohexidine®, alcohol-free essential oil-containing MC® mouth rinse had the same effect on S. mutans counts, higher than the 1% alcohol solution on teeth surface. They had the ability to maintain the S. mutans counts at the same level for 4 days in patients who did not perform any mechanical oral hygiene regimen.

Key words: Chlorhexidine, essential oil, mouth rinses, orthodontics, Streptococcus mutans

INTRODUCTION

Favorable skeletal and dental improvements are often achieved through orthodontic therapy with fixed appliances, although some undesirable side-effects to enamel may also result. If oral hygiene is inadequate, areas of demineralization, called “white spot lesions,” can appear. According to the acidogenic theory, the development of demineralization areas results from the increase in Streptococcus mutans (S. mutans), Streptococcus sobrius, lactobacilli, and actinomyces bacteria, which produce acid around the braces as they metabolize sugar. Two to three weeks after plaque accumulation, white spot lesions appear on the buccogingival areas and may lead to patient dissatisfaction at the end of orthodontic treatment. If these lesions progress to decay, cosmetic or extensive dental interventions are needed.

Some precautions can be taken to decrease the risk of demineralization and to strengthen the enamel structure. A common strategy to improve mechanical plaque removal is to incorporate a chemotherapeutic agent, such as an antibacterial mouthrinse, into the oral hygiene regimen. Mouth rinses that contain essential oils effectively decrease the total number of microorganisms, such as S. mutans. Considerable clinical trial evidence

How to cite this article: Ulkur F, Arun T, Ozdemir F. The effects of three different mouth rinses in a 4-day supragingival plaque regrowth study. Eur J Dent 2013;7:352-8.

Copyright © 2013 Dental Investigations Society. DOI: 10.4103/1305-7456.115420
is available to show that oral hygiene is significantly improved when a mouth rinse containing essential oils, e.g., Listerine® (Johnson and Johnson, McNEIL-PPC, Inc., Skillman, NJ), which contains alcohol or another option as Mouthwash Concentrate® (One Drop Only GmbH, Stieffring, 14, 13627 Berlin – Germany) which is an alcohol free mouthrinse.

The clinical benefits of such mouth rinses are attributable to their bactericidal properties, which prevent or reduce supragingival plaque and gingivitis and decrease intrinsic malodor.[13] Chlorhexidine (CHX)-containing mouth rinses are accepted as the gold standard and are used as a positive control in most trials.[14,15] However, these rinses have side effects, such as enamel discoloration, mucosal erosion, taste disturbance, mouth burning, dry mouth, carcinogenic effects and the smoothing of composite materials, which limits their usage to approximately 5 weeks.[16] To prevent these side effects, alcohol-free CHX mouth rinses have been developed, one of them is Ondrohexidine® (One Drop Only GmbH, Stieffring, 14, 13627 Berlin-Germany), which is an alcohol free mouthrinse.

The purpose of this study was to compare the efficacies of three different mouthrinses in reducing S. mutans colony counts: Ondrohexidine®, Listerine®, and Mouthwash Concentrate®, the latter has not yet been compared to any other mouth rinses in a clinical trial.

MATERIALS AND METHODS

The study was conducted in 70 adults (46 females and 24 males) who were receiving orthodontic treatment.

Each volunteer was selected according to the following criteria: (i) good general health, (ii) no sign of destructive periodontal disease and no more than 4 mm gingival pocket depth, (iii) a minimum of 24 teeth, (iv) no antibiotic treatment during a 3-month period prior to the start of the trial, (v) no regular medication with anti-inflammatory compounds, (vi) no use of tobacco products, (vii) no pregnancy, (ix) no history of allergy to oral care products, (x) no regular use of oral antiseptics, (xi) no fluoride varnish application and (xii) no other dental treatment except orthodontic treatment. The patients had standard edgewise braces on their incisors, canines and premolars and standard edgewise bands on molar teeth. All subjects received written and verbal descriptions of the study design and signed informed consent forms. The study was approved by the regional Ethics Committee.

The subjects were randomly assigned to four test groups. On the odd numbered days of the month, the patients with the odd protocol numbers were assigned to the control group, and the even protocol numbers were assigned to Ondrohexidine® group. On the even numbered days of the month, the patients with odd protocol numbers were assigned to the Listerine® group, and the even protocol numbers were assigned to the Mouthwash Concentrate® group. The negative control group and the three test groups had braces for the last 2-6 months on both the lower and upper dental arches. The control group consisted of 10 patients while the other three test groups consisted of 20 patients each. The plaque on the teeth surface was colored using the plaque disclosing agent Eviplac® (Parana, Brazil), and patients with plaque indexes (PI) between 0 and 1.5 were selected [Figure 1]. PI was determined according to the criteria of the modified Quigley and Hein Plaque Index[17] (on a scale of 0-5). The patients received professional scaling and polishing to remove all the visible plaque, stains, and calculus.

Following professional mechanical cleaning, pellicle samples were collected from the surface between the braces and gingiva from the upper right central incisor, upper right first premolar, lower left central incisor, and lower left first premolar teeth using a microbrush [Figure 2]. The samples from the four quadrants were then spread on square-headed plaque strips from the Dentocult®SM Strip mutans kit (Orion Diagnostica Oy, FI-02101 Espoo, Finland) [Figure 3a]. Then, subjects chewed paraffin gum to increase salivary flow, and saliva samples were collected from the surface of the tongue. These samples were spread on the round-headed Dentocult® Strip [Figure 3b], clipped with plaque strips and placed into tubes containing bacitracin [Figure 3c].

Prior to the trials, patients were informed of the design and limits of the study and instructed accordingly; these instructions included the type, amount, and usage frequency of the mouth rinse. They were also told not to perform any means of mechanical cleaning or to consume any chewing gum or similar products. This was a double-blind study, and the direction and distribution of experimental materials was performed by a secondary clinician. The tests were conducted based on a 4-day plaque accumulation period.[18] The first group of patients constituting the positive control group were directed to use 20 mL of essential oil-containing Listerine® mouth rinse twice a day for 30 s. Listerine® mouth rinse contains eucaliptol (0.092%), menthol (0.042%), methyl salicylate (0.060%), and thymol (0.064%) as
Ulkur, et al.: Three mouthrinses and Streptococcus mutans levels

active ingredients. Inactive ingredients include, water, alcohol (26.9%), benzoic acid, poloxamer 407, sodium benzoate, and caramel. The second group was directed to use 10 mL of 0.1% Ondrohexidine® mouth rinse twice a day for 30 s. The active ingredients of this alcohol-free mouth rinse are essential oil, water, menthol, thymol, eugenol, benzyl benzoate, and potassium hydroxide, with thyme and sage for flavor. The final group was designated as the negative control group and was directed to use 30 mL of 1% hydroalcohol solution 3 times a day for 30 s. The last rinse was performed in the evening of day 4. At the end of the test period, saliva, and plaque samples were collected in an identical fashion to the initial samples on the morning of the 5th day. Both sets of samples were analyzed for comparison. A total of 140 samples were tagged and kept in an incubator at 37°C for 96 h. According to the strip kit manufacturer, the incubation time should be 48 h; however, to avoid the lack of expression of S. mutans colonies, the manufacturer also advised to wait 96 h and re-evaluate the colony counts. Following incubation, S. mutans colony numbers were evaluated on a population density scale from 0 to 3 using the plaque and saliva templates included in a Dentocult® kit. The number of colony-forming units (CFU/mL) with characteristic morphology was screened and scored between 0 and 3. A score of 0 corresponded to zero CFU/mL (S. mutans below detection level); a score of 1 indicated 1-10 CFU and corresponded to approximately <10^4-10^5 CFU/mL; a score of 2 represented 10-100 CFU and corresponded to approximately to 10^5-10^6 CFU/mL; and a score of 3 represented more than 100 CFU and corresponded to more than 10^6 CFU/mL. The evaluation was blinded. One examiner gave the samples to the other without showing the sample names and recorded the scores. Each examiner repeated the scoring for inter- and intra-examiner scoring evaluation.

Statistical analysis
Inter- and intra-examiner reliability was evaluated by kappa (κ) analysis. The significance level was set at P < 0.05.

The test results from the 1st and 2nd sampling days were analyzed separately for each group. The analyses were conducted to compare the effects of 3 different mouth rinses with respect to their efficacy in reducing S. mutans colony numbers. Statistical calculations were performed with the NCSS 2007 Statistical Software (Number Cruncher Statistical System, Utah, USA) program for Windows. In addition to standard descriptive statistical calculations (mean, standard deviation, median and inter-quartile range), the Kruskal-Wallis and ANOVA tests were used for the group comparisons, and post hoc Dunn’s multiple
comparison test was utilized for the two group comparisons. The Wilcoxon test was employed for the assessment of values from the 1st and 4th days. The Chi-square test was performed for the evaluation of qualitative data. The statistical significance level was set at $P < 0.05$.

RESULTS

Intra-examiner reliability in the scoring of $S. mutans$ colony counts from the tooth surface ($k_w$: 0.760, $P = 0.0001$) and tongue surface ($k_w$: 0.790, $P = 0.0001$) was achieved, as well as inter-examiner reliability in the scoring of $S. mutans$ colony counts from the tooth surface ($k_w$: 0.615, $P = 0.0001$) and tongue surface ($k_w$: 0.814, $P = 0.0001$). The patients who were randomly assigned to four groups were found to be balanced with respect to age ($P = 0.251$) and gender ($P = 0.699$) [Table 1].

Statistical analysis showed no significant difference in the group comparison of $S. mutans$ colony count levels from the tooth surface in the first sample ($P = 0.700$). $S. mutans$ colony count levels from teeth surfaces in the second sample showed a statistically significant difference ($P = 0.02$) between groups. The $S. mutans$ colony count level of the second sample of the MC® group ($P = 0.015$) and control group ($P = 0.042$) were found to be significantly higher than those of the first sample, while the Ondrohexidine® and Listerine® groups did not present any significantly different results between the 2 time points ($P = 0.114, P = 0.307$) [Table 2].

The $S. mutans$ colony count level of the control group was significantly higher than those of the Ondrohexidine®, Listerine® and MC® groups ($P = 0.015, P = 0.006, P = 0.048$, respectively) [Table 3]. There was no difference between the groups with regard to the $S. mutans$ colony count levels from the tongue surface in the first sample ($P = 0.110$) [Table 4]. The comparison of the $S. mutans$ colony count levels from the tongue surface of the second samples of the Ondrohexidine®, Listerine® and control groups did reveal statistically significant differences ($P = 0.017$). The two-group comparison revealed that the $S. mutans$ colony count level of the control group was significantly higher than those of the MC®, Listerine® and Ondrohexidine® groups, whereas the comparisons between other groups did not reveal any statistically significant differences ($P > 0.05$), [Table 5].

DISCUSSION

During fixed orthodontic therapy, braces, bands, wires, and other attachments make it difficult for the patient to perform mechanical oral hygiene procedures; this difficulty results in plaque accumulation, which is the main cause of demineralization. Following the placement of fixed appliances, plaque volume, and $S. mutans$ colony counts were shown to increase; after the removal of the appliances, $S. mutans$ counts decreased to normal values. Ulkur, et al. concluded that the most notable etiologic factor contributing to decay formation was $S. mutans$ because this bacterium

### Table 1: Results ($P$ values) of comparison of age and gender distribution of patients into 4 mouth rinse groups

| Age (years) | MC® (15.30±2.34) | Ondrohexidine® (16.75±3.81) | Listerine® (17.15±3.37) | Control (15.80±2.30) | $P=0.251$ |
|-------------|------------------|---------------------------|------------------------|-----------------------|-----------|
| Gender      |                  |                           |                        |                       |           |
| Male        | 9                | 45.0%                     | 6                      | 30.0%                 | 3         | 30.0% | $\chi^2: 1.42$ |
| Female      | 11               | 55.0%                     | 14                     | 70.0%                 | 7         | 70.0% | $P=0.699$ |

$\chi^2$: Chi-square, Significance level $P<0.05$

### Table 2: Streptococcus mutans colony count levels from the teeth surface on the first day after professional cleaning (first sample) and after 4th day (second sample): Results ($P$ values) of Kruskal Wallis test * for comparison of mouth rinses

| Teeth surface | MC®          | Ondrohexidine® | Listerine® | Control | $P^*$ |
|---------------|--------------|---------------|------------|---------|-------|
| First sample  | Median (IQR)* | 0.38 (0-1.25) | 0 (0-1.25) | 0.5 (0-1.44) | 0.84 (0-2) | 0.700 |
|               | Mean±SD†     | 0.7±0.82      | 0.68±0.95  | 0.75±0.86 | 1±0.94 |
| Second sample | Median (IQR)* | 1.18 (0.65-2) | 0.75 (0.25-1.5) | 0.63 (0-1.75) | 2 (1.44-2.31) | 0.02* |
|               | Mean±SD†     | 1.38±0.87     | 0.94±0.8   | 0.88±0.85 | 1.8±0.87 |

$^*$Significance level $P<0.05$, IQR*: Interquartile range, SD*: Standard deviation
produced more acid than *sobrius* and *mitis*. There is double risk with respect to caries formation for orthodontic patients; besides the problem of cleaning of the tooth surfaces, *S. mutans* also exhibit high adhesion to the surfaces of brackets.[23]

Antimicrobial mouth rinses are easy to use with fixed orthodontic appliances, and their ability to access most areas, including embrasures and around the appliances, makes them useful.[9,23] Based on current practices daily rinsing with a 0.05% sodium fluoride mouth rinse[24,25] is recommended for these patients. However patients’ compliance with these directions is often unsatisfactory.[26] There are some undesirable side effects[16] related to the continuous use of antimicrobial mouth rinses; which is why patients are advised to use these mouth rinses on a short-term basis. Also, the clinicians are prompted to seek alternative mouth rinses that could be used for long spans of time. Essential oil (EO)-containing mouth rinses have been shown to produce a significant reduction in plaque endotoxin activity after long-term use.[27] In this context, the present study was designed to compare the effects of three different mouth rinses in the reduction of *S. mutans* colony numbers: Alcohol-free EO mouth rinse, alcohol-containing EO mouth rinse, and alcohol-free 0.1% CHX mouth rinse.

Table 3: *Post hoc* Dunn’s multiple comparison tests to compare the differences between the teeth surface second sample values of the mouth rinses

| Tooth surface/second sample | MC®/ONDROHEXIDINE® | 0.112 |
|----------------------------|--------------------|-------|
| MC®/LISTERINE®            | 0.082              |
| MC®/Control               | 0.048*             |
| Ondrohexidine®/Listerine® | 0.734              |
| Ondrohexidine®/Control    | 0.015*             |
| Listerine®/Control        | 0.006*             |

*Significance level *P*<0.05

Table 4: *Streptococcus mutans* colony counts levels from the tongue surface on the 1st day after professional cleaning (first sample) and after 4th day (second sample): Results (*P* values) of Kruskal Wallis test * for comparison of mouth rinses

| Tongue surface          | MC®     | Ondrohexidine® | Listerine® | Control   | *P*  |
|-------------------------|---------|----------------|------------|-----------|------|
| First sample            |         |                |            |           |      |
| Median (IQR)            | 0 (0-1.75) | 0 (0-2) | 1 (0-2) | 2 (0.75-2.25) | 0.110 |
| Mean±SD                 | 0.75±0.97  | 0.75±1.12      | 1.1±1.02  | 1.6±1.07  |      |
| Second sample           |         |                |            |           |      |
| Median (IQR)            | 0 (0-1.75) | 0 (0-1) | 0.5 (0-1.25) | 2 (1-2.25) | 0.017*|
| Mean±SD                 | 0.8±1.06   | 0.5±0.83       | 0.78±0.94 | 1.7±0.95  |      |
| *P*                     | 0.999    | 0.157          | 0.301    | 0.705     |      |

*Significance level *P*<0.05, IQR*: Interquartile range, SD*: Standard deviation

Although some studies have stated that alcohol showed only a slight antibacterial efficacy against oral bacteria,[28-30] most of the mouth rinse preparations available contain alcohol in various amounts. While alcohol is used as a vehicle to dissolve and stabilize ingredients,[31] to prolong shelf life and as an antiseptic agent, some studies have reported that the presence of alcohol, especially, at high concentrations, results in increases in the effectiveness of mouth rinse solutions.[32,33] However, there are also other studies that report opposite conclusions.[33,34] A study by Van Strydonck *et al.*[35] concluded that a non-alcoholic mouth rinse containing CHX and 0.05% cetyl pyridinium chloride acted similarly to alcohol-containing 0.2% CHX mouth rinse with respect to their effects on plaque inhibition.

CHX mouth rinses are the gold standard for the inhibition of plaque formation,[14,15] however, most of these effective formulations have a high alcohol content,[16] and they are, consequently, inappropriate for some patients.[35-38] High amounts of alcohol and CHX are generally associated with side-effects.[66] As clinicians, we would prefer to recommend mouth rinses that do not have these side effects. Therefore, there is a need to identify alternative, alcohol-free solutions for sensitive patients and those who do not wish to use alcohol-containing mouth rinses, such as former alcoholics or those whose religions prevent the consumption of alcohol.

The alcohol-free EO solution in this study was tested against a negative control, as well as a positive CHX and a benchmark control, as in the study by Rosin *et al.*[39] Alcohol-containing EO mouth rinse was chosen as the benchmark control because the effectiveness of this EO-containing mouth rinse in controlling plaque and reducing the number of colonies of *S. mutans* has been demonstrated in many clinical trials.[18,40-46]
In this study, the alcohol-containing EO mouth rinse was found to be effective while the alcohol-free EO rinse was not effective in inhibiting the \textit{S. mutans} levels on tooth surfaces [Table 2]. The 2 EO mouth rinses contained different EO ingredients; therefore, it was not possible to discuss the effects of alcohol with regard to these two mouth rinses.

The alcohol-free solutions used in this study included a CHX solution and an EO solution. Although the CHX solution used in this study is alcohol-free, it still maintained the \textit{S. mutans} CFU effectively [Tables 2 and 4].

In the 4 days of the non-brushing period, the \textit{S. mutans} levels on tooth surfaces were maintained in groups in which alcohol-free CHX and alcohol-based EO-containing mouth rinses were used [Table 2]. The effect was similar for the two groups. The findings of this study, which involved alcohol-based EO mouth rinse and CHX, are consistent with those of other studies.\cite{39,46} In a study by Riep \textit{et al.}\cite{46} both 0.1% CHX-and alcohol-based EO, which were used as a 20 mL rinse for 30 s, were significantly superior to placebo and exhibited similar performances. Similarly, in the study by Rosin \textit{et al.}\cite{39} a 0.12% Polyhexamethylene Biguanide (PHMB) mouth rinse was compared with a negative control placebo rinse (10% ethanol, flavor), a positive control 0.12% CHX rinse that did not contain alcohol, and a mouth rinse that contained EO and alcohol. Two similar 6-month controlled clinical studies also demonstrated that the EO mouth rinse and the CHX mouth rinse had comparable antiplaque and antigingivitis activities.\cite{45,46}

In contrast, in a study by Moran \textit{et al.}\cite{19} plaque scores with alcohol-based EO mouth rinses were significantly higher than with CHX; however, the dosage regimen of a 1 min rinse with 10 mL of the 0.2% CHX product as opposed to 30 s with 20 mL of alcohol-based EO mouth rinse might have been the reason for the high scores for the CHX mouth rinse in the direct comparison. Among the reasons for the inconsistency in the results and limitations of the studies are the uncontrolled mechanical cleaning procedures incorporated into the study methods, as well as the atypical prescriptions for the use of the mouth rinses.

There were some limitations of this study, which was the exclusion of patients who did not use the mouth rinses as notified in the prescriptions. Some other patients had no \textit{S. mutans} colonization because of high salivary buffer capacity, pH or flow rate.

The findings of this \textit{in vitro} study emphasize as clinical relevance that patients undergoing fixed orthodontic therapy may use mouth rinse in addition to a mechanical oral hygiene regimen to reduce the colony counts of \textit{S. mutans}. Long-term studies should be performed antioxidant to mechanical oral hygiene procedures to ascertain the benefits of essential oil and alcohol free mouth rinses.

**CONCLUSIONS**

Essential oil-containing mouth rinses with and without alcohol, as well as CHX-containing mouth rinses, have the ability to maintain the \textit{S. mutans} counts of the teeth at the same levels in patients who did not perform any mechanical oral hygiene regimen for 4 days.

The mouth rinses used in our study have the ability to maintain the \textit{S. mutans} colony numbers at the same level on the tongue, even though no mechanical oral hygiene regimen is performed.

**ACKNOWLEDGMENTS**

We wish to acknowledge the financial support given by Turkish Scientific and Technical Research Council (TUBITAK). This study was supported by Short-Term R and D Funding Program (1002), 109S193, 2009-2010. We also wish to thank Associate Professor Mustafa Ozyurt from Department of Clinical Microbiology, Gulhane Military Medical Academy for his help in the preparation and for the proofreading of the manuscript.

**REFERENCES**

1. Atack NE, Sandy JR, Addy M. Periodontal and microbiological changes associated with the placement of orthodontic appliances. A review. J Periodontol 1996;67:78-85.

2. Sukontapatipark W, el-Agroudi MA, Selliseth NJ, Thurold K, Selvig KA. Bacterial colonization associated with fixed orthodontic appliances. A scanning electron microscopy study. Eur J Orthod 2001;23:475-84.

| Post hoc Dunn’s multiple comparison test | Tongue surface/second sample |
|-----------------------------------------|------------------------------|
| MC®/Ondrohexidine®                     | 0.389                        |
| MC®/Listerine®                         | 0.924                        |
| MC®/Control                            | 0.025*                       |
| Ondrohexidine®/Listerine®              | 0.307                        |
| Ondrohexidine®/Control                 | 0.002*                       |
| Listerine®/Control                     | 0.021*                       |

*significance level P<0.05
Ulkur, et al.: Three mouthrinses and *Streptococcus mutans* levels

3. Gwinnett AJ, Ceen RF. Plaque distribution on bonded brackets: A scanning microscope study. Am J Orthod 1979;75:667-77.
4. 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.
5. Mitchell L. Decalcification during orthodontic treatment with fixed appliances. Arch Oral Biol 1990;35:199-205.
6. Gorelick L, Geiger AM, Gwinnett AJ. Incidence of white spot formation after bonding and banding. Am J Orthod 1982;81:93-8.
7. Artun J, Brobakken BO. Prevalence of carious white spots after orthodontic treatment with multibonded appliances. Eur J Orthod 1986;8:229-34.
8. Boyd RL. Enhancing the value of orthodontic treatment: Incorporating effective preventive dentistry into treatment. Am J Orthod Dentofacial Orthop 2000;117:601-3.
9. Eley BM. Antibacterial agents in the control of supragingival plaque: A review. Br Dent J 1999;186:286-96.
10. Scheie AA. Modes of action of currently known chemical antiplaque agents other than chlorhexidine. J Dent Res 1989;68:1609-16.
11. White DJ, Barker ML, Klukowska M. In vitro antiplaque efficacy of combined antimicrobial dentifrice and rinse hygiene regimens. Am J Dent 2008;21:189-96.
12. Fine DH, Markowitz K, Furgang D, Goldsmith D, Charles CH, Lisante TA, et al. Effect of an essential oil-containing antimicrobial mouthrinse on specific plaque bacteria in vivo. J Clin Periodontol 2007;34:652-7.
13. Balenseifen JW, Madonia JV. Study of dental plaque in orthodontic patients. J Dent Res 1970;49:320-4.
14. Kocak MM, Ozcan S, Kocak S, Topuz O, Ertan H. Comparison of the efficacy of three different mouthrinse solutions in decreasing the level of *Streptococcus mutans* in saliva. Eur J Dent 2009;3:57-61.
15. Wigger-Albertini W, Gysen K, Asmam EM, Wilhelmi KP. Efficacy of a new mouthrinse formulation on the reduction of oral malodour in vivo. A randomized, double-blind, placebo-controlled, 3-week clinical study. J Breath Res 2010;4:017102.
16. Moran J, Addy M, Kohut B, Hovlias CA, Newcombe RG. Efficacy of mouthrinses in inhibiting the development of supragingival plaque over a 4-day period of no oral hygiene. J Periodontol 1994;65:904-7.
17. Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. J Am Dent Assoc 1962;65:26-9.
18. Moran J, Addy M, Newcombe R. A 4-day plaque regrowth study comparing an essential oil mouthrinse with a triclosan mouthrinse. J Clin Periodontol 1997;24:636-9.
19. Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. J Am Dent Assoc 1962;65:26-9.
20. Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. J Am Dent Assoc 1962;65:26-9.
21. De Soet JJ, Nyyvd B, Killian M. Stain-related acid production by oral streptococci. Caries Res 2000;34:486-90.
22. Fournier A, Payant L, Bouclin R. Adherence of *Streptococcus mutans* to orthodontic brackets. Am J Orthod Dentofacial Orthop 1998;114:414-7.
23. Santos A. Evidence-based control of plaque and gingivitis. J Clin Periodontol 2003;30 Suppl 5:13-6.
24. Benson PE, Parkin N, Millett DT, Dyer FE, Vine S, Shah A. Fluorides for the prevention of white spots on teeth during fixed brace treatment. Cochrane Database Syst Rev 2004;3:CD00809.
25. Benson PE, Shah AA, Millett DT, Dyer F, Parkin N, Vine RS. Fluorides, orthodontics and demineralization: A systematic review. J Orthod 2005;32:102-14.
26. Geiger AM, Gorelick L, Gwinnett AJ, Benson BJ. Reducing white spot lesions in orthodontic populations with fluoride rinsing. Am J Orthod Dentofacial Orthop 1992;101:403-7.
27. Fine DH, Letizia I, Mandel JD. The effect of rinsing with Listerine antiseptic on the properties of developing dental plaque. J Clin Periodontol 1985;12:660-6.
28. Germo P, Baastad KL, Rölla G. The plaque-inhibiting capacity of 11 antibacterial compounds. J Periodontal Res 1970;5:102-9.
29. Myklebust S. Comparative antibacterial effectiveness of seven hand antiseptics. Scand J Dent Res 1985;93:546-54.
30. Sisson CH, Wong L, Cutress TW. Inhibition by ethanol of the growth of biofilm and dispersed microcosm dental plaques. Arch Oral Biol 1996;41:27-34.
31. Claffey N. Essential oil mouthwashes: A key component in oral health management. J Clin Periodontol 2003;30 Suppl 5:224-2.
32. Wikén Albertsson K, Persson A, Lingström P, van Dijken JW. Effects of mouthrinses containing essential oils and alcohol-free chlorhexidine on human plaque acidogenicity. Clin Oral Investig 2010;14:107-12.
33. Herrera D, Roldán S, Santacruz I, Santos S, Masdevall M, Sanz M. Differences in antimicrobial activity of four commercial 0.12% chlorhexidine mouthrinse formulations: An *in vitro* contact test and salivary bacterial counts study. J Clin Periodontol 2003;30:307-14.
34. 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;28:1127-36.
35. Van Struydonck DA, Timmerman MF, van der Velden U, van der Weijden GA. Plaque inhibition of two commercially available chlorhexidine mouthrinses. J Clin Periodontol 2005;32:305-9.
36. Mankodi S, Baurothy K, Witt JJ, Boulo S, He T, Gibb R, et al. A 6-month clinical trial to study the effects of a cetylpyridinium chloride mouthrinse on gingivitis and plaque. Am J Dent 2005;18:93-14A.
37. Witt JJ, Walters P, Boul S, Gibb R, Dunavent J, Futt M. Comparative clinical trial of two antigingivitis mouthrinses. Am J Dent 2000;13:15-17A.
38. White DJ. An alcohol-free therapeutic mouthrinse with cetylpyridinium chloride (CPC) – The latest advance in preventive care: Crest Pro-Health Rinse. Am J Dent 2005;18 Spec No: 3-8A.
39. Rosin M, Welk A, Kocher T, Majic-Todd A, Kramer A, Pfitzen 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 regrowth. J Clin Periodontol 2002;29:392-9.
40. Lamster IB, Alfano MC, Seiger MC, Gordon JM. The effect of Listerine Antiseptic on reduction of existing plaque and gingivitis. Clin Prev Dent 1983;5:12-6.
41. Gordon JM, Lamster IB, Seiger MC. Efficacy of Listerine antisepic in inhibiting the development of plaque and gingivitis. J Clin Periodontol 1985;12:697-704.
42. Axelsson P, Lindhe J. Efficacy of mouthrinses in inhibiting dental plaque and gingivitis in man. J Clin Periodontol 1987;14:205-12.
43. DePaola LG, Overholser CD, Meiller TF, Minah GE, Niehaus C. Chemotherapeutic inhibition of supragingival dental plaque and gingivitis development. J Clin Periodontol 1989;16:311-5.
44. Ross NM, Charles CH, Dills SS. Long-term effects of Listerine antiseptic on dental plaque and gingivitis. J Clin Dent 1989;1:92-5.
45. Overholser CD, Meiller TF, DePaola LG, Minah GE, Niehaus C. Comparative effects of 2 chemotherapeutic mouthrinses on the development of supragingival dental plaque and gingivitis. J Clin Periodontol 1990;17:575-9.
46. Riep BG, Bernimoulin JP, Barnett ML. Comparative antiplaque effectiveness of an essential oil and an amine fluoride/stannous fluoride mouthrinse. J Clin Periodontol 1999;26:164-8.