Effect of vegetable oil (Brazil nut oil) and mineral oil (liquid petrolatum) on dental biofilm control

Cíntia de Fátima Buldrini Filogônio[a]
Rodrigo Villamarim Soares[a]
Martinho Campolina Rebello Horta[a]
Cláudia Valéria de Sousa Resende Penido[a]
Roberval de Almeida Cruz[a]

[a] Departamento de Odontologia, Pontifícia Universidade Católica de Minas Gerais, Belo Horizonte, MG, Brazil.

Abstract: Dental biofilm control represents a basic procedure to prevent caries and the occurrence of periodontal diseases. Currently, toothbrushes and dentifrices are used almost universally, and the employment of good oral hygiene allows for appropriate biofilm removal by both mechanical and chemical control. The aim of this study was to evaluate the effectiveness of adding vegetable or mineral oil to a commercially available dentifrice in dental biofilm control. A comparison using the Oral Hygiene Index Simplified (OHI-S) was performed in 30 individuals who were randomly divided into three groups. Group 1 (G1) received a commercially available dentifrice; the composition of this dentifrice was modified by addition of mineral oil (Nujol®) for group 2 (G2) or a vegetable oil (Alpha Care®) for group 3 (G3) at 10% of the total volume, respectively. The two-way repeated-measures analysis of variance (two-way ANOVA) was used to test the effect of group (G1, G2 and G3) or time (baseline, 45 days and 90 days) on the OHI-S index scores. Statistical analysis revealed a significant reduction in the OHI-S at day 90 in G2 ($p < 0.05$) and G3 ($p < 0.0001$) in comparison to G1. Therefore, the addition of a vegetable or a mineral oil to a commercially available dentifrice improved dental biofilm control, suggesting that these oils may aid in the prevention and/or control of caries and periodontal disease.

Descriptors: Dental Plaque; Mineral Oil; Plant Oils; Toothbrushing; Toothpastes.

Introduction

Dental biofilm control is the primary aim in caries and periodontal disease prevention. Mechanical removal of the biofilm, performed by appropriate use of toothbrush and dental floss, has been the main tool in oral hygiene care. There is a direct relationship between oral hygiene level, the quantity and quality of dental biofilm, and disease prevalence and severity. However, the prevalence of caries and periodontal disease among the population indicates that a significant number of individuals do not exhibit adequate biofilm control.[1]

A previous study recommended brushing twice daily complemented by inter-dental cleaning with dental floss.[2] Subsequently, studies have indicated that disease control was improved when instructions regarding biofilm control were more frequently offered to individuals.[3] This strategy combined with chemical agents in regular oral hygiene programs has

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding author:
Roberval de Almeida Cruz
E-mail: *roberval@pucminas.br*

Received for publication on Jun 08, 2011
Accepted for publication on Oct 11, 2011

been proposed because the isolated use of mechanical procedures is not considered ideal for dental biofilm control.[4] Despite recognition of the effectiveness of mechanical biofilm control, the process may be potentialized by chemical control, especially in individuals at high risk to develop oral diseases.[5,6]

The chemical control of dental biofilms had been studied for several years. The effect of procedures and oral hygiene products on removal of organic deposits from dental surfaces has also been investigated. The ability of different agents to reduce bacterial adhesion and to inhibit microbial growth on the oral environment have been reported.[7] In addition to the ability for biofilm removal without causing damage, abrasion or rugosity in the dental enamel, chemical agents have exhibited the capacity to selectively eliminate oral pathogens without provoking a negative impact in commensal microbiota.[8]

A small number of antimicrobial agents have shown clinical efficacy in dental biofilm control due to the inherent problems in the activity of agents within the oral environment and the difficulties incorporating these agents into the dental products.[9] Although the use of chemical agents with oral hygiene mechanical procedures is a primary goal in dentistry, analysis of the clinical benefits and potential adverse effects of these compounds on dental biofilm ecology has also been conducted.[10] In particular, mouthrinses, toothpastes, and other topical solutions are suitable vehicles for dental biofilm control agents. However, the use of the rinse or gel in combination with regular toothbrushing with fluoride toothpaste results in associated costs that are higher than the equivalent to using an antibiofilm dentifrice.[11]

Because dentifrice use has exhibited effectiveness in dental biofilm control, different formulations have been proposed to improve this process. A previous study reported that the addition of certain oils to the dentifrices provided adequate anti-dental biofilm and anti-gingivitis action.[9] The proposed mechanism indicated that a protective barrier on the dental enamel generated by fatty oils interfered with dental biofilm formation. Another study reported that the addition of an essential oil to mouthrinse did not interfere with fluoride activity against biofilm acidogenicity.[12]

Different oils, usually obtained from a mineral or vegetable source, are commercially available and are classified into two main groups (i.e., fixed – fatty; volatile – essential).[13] Essential oils are used in medicine, cosmetics and the food industry, and their volatile components possess antimicrobial, antifungal and insecticide activity.[14] The activity of essential oils in dental biofilm inhibition and reduction has been reported,[15] and these properties are derived from the absorption to dental enamel that results in a hydrophobic surface and reduces dental biofilm deposition.[16]

Favorable antibacterial activities of two essential oils *(Mentha piperita* and *Cuminum cyminum*) against *Streptococcus mutans* and *Streptococcus pyogenes* were recently reported. This effect was observed in volunteers brushing their teeth with dentifrices containing the essential oils.[17] Ginger oil has also been shown to reduce the dental biofilm formation rate and the total number of aerobic microorganisms colonies in adolescents.[18]

Mineral oils added to dentifrices resulted in new formulations that were patented. For instance, there is a water-free dentifrice, containing mineral oils, that provides acceptable viscosity characteristics, suitable extrudable consistency and user-acceptance due the absence of an undesirable oily or greasy appearance.[19] Another dentifrice with mineral oil (petrolatum) was used for dental biofilm reduction and periodontal disease control.[20] Other dentifrice formulations containing mineral oils have been indicated for the prevention and control of caries due to properties related to dental biofilm debridement, enamel remineralization, microbial adherence inhibition, and salivary pH maintenance or increase.[21]

Thus, dental biofilm control is an important component in the prevention of caries and periodontal disease. In addition, chemical agents, such as oils, exhibit properties that improve dental biofilm control. Therefore, the aim of this study was to evaluate the effectiveness of adding vegetable or mineral oil to a commercially available dentifrice in dental biofilm control.

Methodology

Participants were randomly selected, undergrad-

uate students from the Department of Dentistry who volunteered to participate in the present study. The inclusion criteria were that the participant exhibited good general health and toothbrushing habits (i.e., regular and thorough brushing). The following exclusion criteria were adopted:

- recent (in the past 3 months) use of antibiotics;
- use of orthodontic appliances;
- chronic usage of anti-inflammatory drugs; or
- pregnancy.

A total of 30 individuals were selected. These individuals had an age range of 18 to 21 years old, and there were 23 females and 7 males. The students were randomly divided into three groups, and each group had 10 participants. Group 1 (G1) received an original dentifrice that is currently available on the market (Colgate Total®, Colgate-Palmolive, Sao Paulo, Brazil). For group 2 (G2), the mineral oil or "liquid petrolatum" (Nujol®, Schering-Plough, Rio de Janeiro, Brazil) was added to the same dentifrice (Colgate Total®) at 10% of the total volume. For group 3 (G3), the vegetable oil or "Brazil nut oil" (Alpha Care®, Vital Âtman, Sao Paulo, Brazil) was also added to the dentifrice (Colgate Total®) at 10% of the total volume. This specific volume of oil (10%) was selected based on previous reports.[22] The dentifrice was repacked (G1) or prepared and repacked (G2 and G3) by a manipulation pharmacy. Dentifrice tubes were labeled to ensure that participants and the researcher analyzing the dental biofilm were blinded to the product content. Patients were warned to stop use of the tested toothpaste and immediately contact the researchers in the occurrence of adverse effects, such as mucosa ulcerations, burning sensation or pain.

Basic fuchsine (1%) solution (BS Pharma, Belo Horizonte, Brazil) was applied with a cotton tip to analyze the dental biofilm, and the excess fuchsine was removed by rinsing with water. The dental biofilm quantification criteria used in this study was the Oral Hygiene Index Simplified (OHI-S).[23] Briefly, six dental surfaces were evaluated:

a. posterior teeth – vestibular surfaces of permanent upper right and left first molar; lingual surfaces of permanent lower right and left first molar; and
b. anterior teeth – vestibular surfaces of permanent upper right central incisor and permanent lower left central incisor.

If one of these dental elements was absent, evaluation was performed in the first tooth located distally. Dental biofilms were classified as follows:

- 0 (zero) - absence of stained dental biofilm;
- 1 - stained dental biofilm covering less than 1/3 of the surface;
- 2 - stained dental biofilm covering more than 1/3, but less than 2/3 of the surface; and
- 3 - stained dental biofilm covering more than 2/3 of surface.

The OHI-S index was calculated using the following formula:

$$\text{OHI-S} = \frac{\text{sum of vestibular and lingual indexes}}{\text{total of examined surfaces}}$$

Participants were asked to refrain from eating or drinking two hours prior to the exam. The exams were conducted in the morning (between 10:00 a.m. and 11:00 a.m.), and in the same dental office by a unique trained (Kappa = 0.76) examiner. OHI-S index scores were analyzed on day one (baseline), before the use of dentifrices for each specific group. After this exam, participants received dentifrice tubes that were sufficient for toothbrushing up to four times daily, and new toothbrushes (Oral B®, Gillete do Brazil, Sao Paulo, Brazil) were also provided to promote standardization. Participants were instructed to maintain their routine oral hygiene habits, and the OHI-S index was reevaluated at 45 and 90 days after the first exam.

OHI-S index scores were initially submitted to the D'Agostino-Pearson normality test, and a normal distribution was detected. Subsequently, the two-way repeated-measures analysis of variance (two-way ANOVA; Bonferroni post hoc analysis) was used to test the effect of each group (G1, G2 and G3) or time (baseline, 45 days and 90 days) on the OHI-S index scores. The level of significance was set at 5%, and statistical analysis was performed using

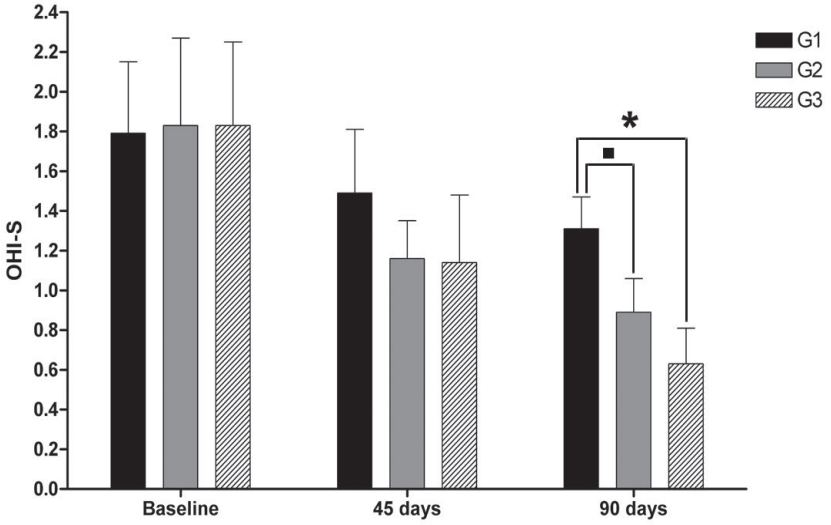

Figure 1 - Mean and standard deviation of OHI-S values for each evaluated group and time.

G1 (control group); G2 (mineral oil group); G3 (vegetal oil group).

■ $p<0.05$; ✱ $p<0.0001$.

p values were obtained by two-way repeated-measures ANOVA (Bonferroni post hoc analysis).

GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, USA).

This study was independently reviewed and approved by the Ethics Committee of PUC Minas. Informed consent was obtained from all individuals prior to their participation, and the rights of these subjects were protected at all times.

Results

The mean and standard deviation values of the OHI-S index scores in each evaluated group and time are illustrated in Figure 1 and are described as follows:

- Day 1 (baseline):
  - 1.79 ± 0.36 (G1),
  - 1.83 ± 0.44 (G2) and
  - 1.83 ± 0.42 (G3);

- 45 days:
  - 1.49 ± 0.32 (G1),
  - 1.16 ± 0.19 (G2) and
  - 1.14 ± 0.34 (G3);

- 90 days:
  - 1.31 ± 0.16 (G1),
  - 0.89 ± 0.17 (G2) and
  - 0.63 ± 0.18 (G3).

The statistical analysis demonstrated that the OHI-S index scores are affected by time ($p < 0.0001$) and by group ($p = 0.0196$). No significant differences in OHI-S index scores ($p > 0.05$) were observed among G1, G2 and G3 at day 1 (baseline). At day 45, no significant differences in OHI-S index scores ($p > 0.05$) were observed among G1, G2 and G3. At the final time point (day 90), significant differences in OHI-S index scores were observed between G1 and G2 ($p < 0.05$) and also G1 and G3 ($p < 0.0001$).

Discussion

The present study revealed the absence of significant differences among groups at baseline, indicating that the groups were homogeneous in previous oral hygiene. Even though the OHI-S index score means of G2 and G3 were lower than G1, the results at day 45 suggest that this amount of time using the tested toothpastes was not sufficient to promote a statistically significant difference in dental biofilm control. In contrast, a 90 day period using the tested toothpastes was sufficient to promote a significant improvement in dental biofilm control in G2 and G3 compared to G1.

The importance of dental biofilm control by daily toothbrushing with dentifrices containing chemical agents (i.e., fluoride) has been previously

reported.[24,25] In this regard, a number of studies have indicated that treatment with only mechanical dental biofilm control might not be sufficient to prevent oral diseases.[3,9,25,26] Therefore, the use of certain agents to improve dental biofilm control should be analyzed.[4-6] Specific dentifrice constituents promote the chemical and mechanical control of dental biofilms. However, new formulations should be developed to improve dental biofilm control and to prevent caries and periodontal diseases prevention.

In the present study, subjects who brushed their teeth with toothpaste supplemented with mineral oil or vegetable oil exhibited reduced oral hygiene indexes. Notably, the absence of differences among groups at baseline demonstrates that the groups were homogeneous in their potential for dental biofilm control. Because previous studies have reported that improved dental biofilm control occurred in patients at the start of evaluations,[2] we decided to analyze OHI-S for 90 days. Our results suggest that insertion of oils in dentifrices may lead to important beneficial effects.

These results are similar to those from a previous study that used almond oil without abrasives[9] and to another study that evaluated the effect of essential oils used as an adjunct to daily toothbrushing without supervision.[27] Therefore, the use of anti-microbial dental agents should be considered as a complementary procedure in dental biofilm control.[4,6]

The properties of essential oils include the capacity to penetrate the biofilm, kill bacteria and reduce dental biofilm volume and pathogenicity,[28] resulting in oral health improvement.[10] The use of oral hygiene products containing oils twice daily reduces the level of specific bacteria associated with gingival diseases.[29] Previous reports have shown that patients with adequate oral hygiene habits do not always manage to appropriately remove dental biofilm; however, the combination of oils with dental procedures might enhance dental biofilm control due to a series of mechanisms, such as inhibition of dental biofilm proliferation, interference in colonization and/or microbicidal activity.[3]

The addition of oils in dentifrices was effective, although supervised toothbrushing to reduce dental plaques and gum bleeding in individuals submitted to prosthetic treatment is still imporant.[9] The addition of oils to dentifrices may reduce costs, due to the replacement of several well-known expensive antibiofilm agents in addition to the simple mechanism that does not require special equipment. Furthermore, drugstore expenses are lower as the active principles in the oil last longer.[13]

In the current study, five volunteers reported discomfort with the taste of the dentifrices containing vegetable oil. Although taste perception was not included in the initial evaluation, it is important to include that these complaints did not occur after 15 days using these specific dentifrices.

To date, the interaction between essential oils and other components of dentifrices is not known, although a previous study demonstrated that these oils do not interfere with fluoride activity against biofilm acidogenicity on mouthrinses.[12] Moreover, future studies should address this issue because the potential cytotoxic effects of the long term use of dentifrices containing Alpha Care® and Nujol® was not investigated in this study.

Finally, participants of the present study were selected among undergraduate dental students. Therefore, this restricted sample is composed of younger and healthier individuals who exhibit better oral hygiene habits and a lower prevalence of oral diseases in comparison to the general population. Therefore, additional studies should evaluate the effectiveness of vegetable and mineral oil addition to dentifrices for dental biofilm control in the general population because these individuals might have a higher prevalence of systemic and periodontal diseases in addition to poor oral hygiene habits.

Conclusions

In this study, the addition of a vegetable or a mineral oil to a commercially available dentifrice improved dental biofilm control. Therefore, additional studies should be conducted to better understand these products containing essential oils. Dentistry professionals should consider and recognize treatments containing essential oils and inform their patients and Public Health Services of the associated benefits.

References

1. Brown LJ, Brunelle JA, Kingman A. Periodontal status in the United States, 1998–91: prevalence, extend and demographic variation. J Dent Res. 1996 Feb;75(Spec Nº):672-83.
2. Sharma N, Charles CH, Lynch MC, Qaqish J, Mcguire JA, Galustians JG, *et al*. Adjunctive benefit of an essential oil-containing mouthrinse in reducing plaque and gingivitis in patients who brush and floss regularly: a six-month study. J Am Dent Assoc. 2004 Apr;135(4):496-504.
3. Stoeken JE, Paraskevas S, van der Weijden GA. The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: a systematic review. J Peridontol. 2007 Jul; 78(7):1218-28.
4. Coelho J, Kohut BE, Mankodi S, Parikh R, Wu MM. Essential oils in an antiplaque and antigingivitis dentifrice: a 6- month study. Am J Dent. 2000 Sep;13(Special nº):5C-10C.
5. Gunsolley JC. Clinical efficacy of antimicrobial mouthrinses. J Dent. 2010 Jun;38(Suppl 1):S6-10.
6. Albert-Kiszely A, Pjetursson BE, Salvi GE, Witt J, Hamilton A, Persson GR *et al*. Comparison of the effects of cetylpyridinium chloride with an essential oil mouthrinse on dental plaque and gingivitis – a six month randomized controlled clinical trial. J Clin Periodontol. 2007 Aug;34(8):658-67.
7. Shimotoyodome A, Koudate T, Kobayashi H, Nakamura J, Tokimitsu I, Hase T, *et al*. Reduction of Streptococcus mutans adherence and dental biofilm formation by surface treatment with phosphorylated polyethylene glycol. Antimicrob Agents Chemother. 2007 Oct;51(10):3634–41.
8. Goodson JM, Palys MD, Carpino E, Regan EO, Sweeney M, Socransky SS. Microbiological changes associated with dental prophylaxis. J Am Dent Assoc. 2004 Nov;135(11):1559-64.
9. Aguiar AA, Saliba NA. Toothbrushing with vegetable oil: a clinical and laboratorial analysis. Braz Oral Res. 2004 Apr-Jun;18(2):168-73.
10. Haffajee AD, Roberts C, Murray L, Veiga N, Martin L, Teles RP, *et al*. Effect of herbal, essential oil, and chlorhexidine mouthrinses on the composition of the subgingival microbiota and clinical periodontal parameters. J Clin Dent. 2009 Jul;20(7):211-7.
11. Cummins D. Vehicles: how to delivery the goods. Periodontol 2000. 1997 Oct;15(10):84-99.
12. Zhang JZ, Harper DS, Vogel GL, Schumacher G. Effect of an essential oil mouthrinse, with and without fluoride, on plaque metabolic acid production and pH after a sucrose challenge. Caries Res. 2004 Nov-Dec;38(6):537-41.
13. Warnke PH, Sherry E, Russo PAJ, Acil Y, Wiltfang J, Sivananthan S *et al*. Antibacterial essential oils malodorous cancer patients: Clinical observations in 30 patients. Phytomedicine. 2006 Jul;13(7):463-7.
14. Tabanca N, Demirci B, Gürbüz I, Demirci F, Becnel JJ, Wedge DE, Başer KH. Essential oil composition of five collections of Achillea biebersteinii from central Turkey and their antifungal and insecticidal activity. Nat Prod Commun. 2011 May;6(5):701-6.
15. Patel RM, Malaki Z. The effect of a mouthrinse containing essential oils on dental plaque and gingivitis. Evid Based Dent. 2008;9(1):18-9.
16. Veeregowda DH, van der Mei HC, Busscher HJ, Sharma PK. Influence of fluoride-detergent combinations on the visco-elasticity of adsorbed salivary protein films. Eur J Oral Sci. 2011 Feb;119(1):21-6.
17. Shayegh S, Rasooli I, Taghizadeh M, Astaneh SD. Phytotherapeutic inhibition of supragingival dental plaque. Nat Prod Res. 2008 Mar;22(5):428-39.
18. Asokan S, Emmadi P, Chamundeswari R. Effect of oil pulling on plaque induced gingivitis: a randomized, controlled, triple-blind study. Indian J Dent Res. 2009 Jan-Mar;20(1):47-51.
19. Mayrand LP, Winston-Sale NC, inventors. Extar Company, assignee. Water-free toothpastes containing metaphosphates, calcium carbonate and mineral oil. US patent 3,475,533. 1969 Oct.
20. Guay CB, Hintonn JP, inventors. The Procter & Gamble Company, assignee. Oral compositions containing monoperoxy acids. US patent 5,192,532. 1993 Mar.
21. Pollock JJ, McNamara TF, inventors. The Research Foundation of State University of New York, assignee. Dental compositions containing monovalent ions. US patent 4,861,582. 1989 Aug.
22. Baar AM, inventor. Method for the oral treatment of dogs and others animals. US patent 4,329,333.1982 May.
23. Broadbent JM, Thomson WM, Boyens JV, Poulton R. Dental plaque and oral health during the first 32 years of life. J Am Dent Assoc. 2011 Apr;142(4):415-26.
24. White DJ, Barker ML, Klukowska M. *In vivo* antiplaque efficacy of combined antimicrobial dentifrice and rinse hygiene regimens. Am J Dent. 2008 Jun;21(3):189-96.
25. Teles RP, Teles FR. Antimicrobial agents used in the control of periodontal biofilms: effective adjuncts to mechanical plaque control? Braz Oral Res. 2009;23(Suppl 1):39-48.
26. Fine DH, Furgang D, Sinatra K, Charles C, McGuire A, Kumar LD. *In vivo* antimicrobial effectiveness of an essential oil-containing mouth rinse 12 h after a single use and 14 days' use. J Clin Periodontol. 2005 Apr;32(4):335-40.
27. Quirynen M. Management of oral malodour. J Clin Periodontol. 2003;30(Suppl 5):17-8.
28. Ouhayoun JP. Penetrating the plaque biofilm: impact of essential oil mouthwash. J Clin Periodontol. 2003;30(Suppl 5):10-2.
29. Fine DH, Markowitz K, Furgang D, Goldsmith D, Ricci-Nittel D, Charles CH, *et al*. Effect of rinsing with an essential oil–containing mouthrinse on subgingival periodontopathogens. J Periodontol 2007 Oct;78(10):1935-42.