Antimicrobial agents used in the control of periodontal biofilms: effective adjuncts to mechanical plaque control?

Abstract: The control of biofilm accumulation on teeth has been the cornerstone of periodontal disease prevention for decades. However, the widespread prevalence of gingivitis suggests the inefficiency of self-performed mechanical plaque control in preventing gingival inflammation. This is particularly relevant in light of recent evidence suggesting that long-standing gingivitis increases the risk of loss of attachment and that prevention of gingival inflammation might reduce the prevalence of mild to moderate periodontitis. Several antimicrobials have been tested as adjuncts to mechanical plaque control in order to improve the results obtained with oral home care. Recent studies, including meta-analyses, have indicated that home care products containing chemical antimicrobials can provide gingivitis reduction beyond what can be accomplished with brushing and flossing. Particularly, formulations containing chlorhexidine, mouthrinses containing essential oils and triclosan/copolymer dentifrices have well documented clinical antiplaque and antigingivitis effects. In vivo microbiological tests have demonstrated the ability of these antimicrobial agents to penetrate the biofilm mass and to kill bacteria growing within biofilms. In addition, chemical antimicrobials can reach difficult-to-clean areas such as interproximal surfaces and can also impact the growth of biofilms on soft tissue. These agents have a positive track record of safety and their use does not seem to increase the levels of resistant species. Further, no study has been able to establish a correlation between mouthrinses containing alcohol and oral cancer. In summary, the adjunct use of chemical plaque control should be recommended to subjects with well documented difficulties in achieving proper biofilm control using only mechanical means.

Descriptors: Dental plaque; Gingivitis; Oils, essential; Triclosan.
Introduction

Since the 1960s when Löe and coworkers\(^1\) established the unequivocal role of dental plaque as the etiological agent of gingivitis, mechanical plaque control has become the cornerstone of periodontal therapy. However, the almost ubiquitous prevalence of gingivitis would suggest that control of periodontal biofilms through mechanical means is inefficient. The difficulty in achieving an “ideal” mechanical plaque control has led scientists and clinicians to seek chemical antimicrobial agents that could help inhibit biofilm formation on tooth surfaces. The current review will concentrate on antimicrobial agents present in formulations of self-care oral hygiene products commercially available in the Brazilian market. The goal is to critically review products available to the Brazilian public regarding their antiplaque and antigingivitis effects. In addition, the effects of these chemical agents on the oral microbiota will be discussed. Important considerations such as the long-term safety of oral antimicrobial agents and the impact of control of gingivitis in the development and progression of more severe forms of periodontal diseases will also be addressed.

Current understanding of the pathogenesis of periodontal diseases indicates that the presence of gingivitis is a pre-requisite for the development of periodontitis.\(^2\) However, it is also well recognized that even long standing severe gingivitis will not necessarily lead to loss of attachment.\(^3\) These observations have led some authors to question the relevance of mild to moderate forms of gingivitis as a public health problem, and if efforts to control this disease at a populational level would be justifiable.\(^4\) Recent data from a 26-year longitudinal study of 565 Norwegian males indicated that sites that bled on probing on every examination (gingival index [GI] = 2) had approximately 70% more attachment loss than non-inflamed sites (GI = 0 at all visits).\(^5\) This study supports the pivotal role of gingivitis as a risk factor for attachment loss and emphasizes the chronic nature of periodontitis, requiring time to progress. Epidemiological data from a Swedish study following randomly selected individuals in different age groups (20-80 years of age) over a 30-year period (1973-2003) suggest that plaque control on a populational level might reduce not only the prevalence of gingivitis but also the prevalence of moderate forms of periodontitis.\(^6\) Subjects were classified according to the severity of their periodontal disease into: 1) periodontal health; 2) gingivitis; 3) moderate periodontitis and 4) and 5) severe or advanced periodontitis. In all age groups the proportion of periodontally healthy subjects increased, while the prevalence of gingivitis and moderate periodontitis decreased. The percentage of individuals in category 4 also tended to decrease, while their number of remaining teeth increased over time. This 30-year cohort study demonstrates that strategies aiming at the control of gingivitis at a populational level could reduce the prevalence of mild to moderate forms of periodontitis.

Limitations of mechanical plaque control

Despite its essential role in the prevention of gingivitis and periodontitis, mechanical plaque control is not properly practiced by most individuals. A survey conducted in the United Kingdom concluded that an average of one-third of teeth in 72% of all dentate adults examined had visible plaque.\(^7\) Brushing techniques are particularly limited in their access to interproximal plaque of premolars and molars, and control of biofilm accumulation on these areas requires additional devices such as dental floss.\(^8\) Surveys conducted in developed countries reveal that the percentage of individuals who claim to use interproximal cleaning devices on a daily basis range from 11 to 51%.\(^9\) A systematic review of the effectiveness of self-performed mechanical plaque removal in adults with gingivitis concluded that the quality of the mechanical plaque control was not sufficiently effective in reducing gingivitis.\(^10\) Taken together, these studies indicate that consistently effective brushing is uncommon, leaving room for improvement. Therefore, the adjunct use of antimicrobial agents might be beneficial for the adjunct control of supragingival biofilms.

An additional limitation of mechanical plaque control procedures is that they concentrate solely on the hard surfaces of the oral cavity. Although the non-shedding surfaces of the teeth provide an excellent surface for the establishment and growth of
biofilms, they represent a relatively small percentage of the total area of the oral cavity (21-23%). Recent studies have demonstrated that microorganisms involved in the etiology of gingivitis and periodontitis accumulate on several soft tissue surfaces of the mouth, which serve as a source of bacteria for the colonization of tooth surfaces. Chemical anti-plaque agents present in mouthrinses or dentifrices could reach these soft tissue surfaces, improving the control of biofilm growth on these surfaces and delaying microbial accumulation on teeth. This mechanism was illustrated in a study that examined the rate of biofilm accumulation on teeth after a 3-week preparatory phase that included oral hygiene instructions and frequent professional cleanings. In the last week of this preparatory phase, the experimental group rinsed and gargled twice a day with a 0.2% chlorhexidine digluconate (CHX) solution and brushed their tongue with a 1% CHX gel once a day. This intensive protocol of control of the soft tissue biofilm resulted in a lower mean plaque index (Turesky modified Quigley-Hein index [QHI]) at days 1, 2 and 4 of undisturbed plaque re-accumulation. Data from checkerboard DNA-DNA hybridization also revealed that the mean total DNA probe count was statistically significantly higher in the control group compared to the experimental group at days 2 and 4 of plaque re-growth. These findings suggest that the daily use of chemical antimicrobial agents that affect the soft tissue biofilms could retard plaque accumulation on tooth surfaces.

### Meta-analysis of antiplaque and antigingivitis agents

A recently published meta-analysis of antiplaque and antigingivitis agents provided strong evidence in favor of the use of antimicrobial agents as adjuncts to mechanical plaque control. A total of 50 manuscripts including 70 active groups met the inclusion criteria and were analyzed. Of the dentifrices examined only formulations containing triclosan/copolymer demonstrated a clinically and statistically significant antiplaque effect. Fourteen of the 18 active groups using triclosan/copolymer had significant decreases in plaque accumulation. The author highlighted two mouthrinses with consistent anti-plaque effects: 0.12% CHX and essential oils-containing formulations (menthol [0.042%], thymol [0.064%], methyl salicylate [0.060%], and eucalyptol [0.092%]). CHX resulted in statistically significant reduction in plaque in all studies reviewed. Only 1 active group out of 25 that used essential oils did not show statistically significant reductions in plaque. The results of studies employing GI to assess gingivitis were statistically and clinically significant for 0.12% CHX mouthrinses (5/6 active groups), dentifrices containing triclosan/copolymer (12/16 active groups) and mouthrinses with essential oils (3/8 active groups). Of the trials using MGI (Modified Gingival Index), 13/17 active groups demonstrated statistically significantly less gingivitis in the groups using essential oils compared to controls. Based on these results we will focus our review on studies examining the clinical and microbiological effects of triclosan/copolymer dentifrices and essential oil mouthrinses. CHX will not be reviewed extensively due to its well known side effects, which preclude its use as a long term adjunct to oral hygiene.

### Effects of essential oil mouthrinses on periodontal biofilms and gingivitis

Essential oils have a broad antimicrobial spectrum, affecting Gram-positive and Gram-negative bacteria and yeasts. The efficacy of oral antiseptics is usually attributed to their bactericidal activity, but essential oils also work by interfering with bacterial colonization of the tooth surface. Essential oils stop bacteria from aggregating with Gram-positive pioneer species, slows bacterial multiplication, and extract endotoxins from Gram-negative pathogens. This can lead to a reduced bacterial load, slow plaque maturation, and decreased plaque mass and pathogenicity. The bactericidal mechanisms of essential oils involve the rupture of the cell wall and enzymatic inhibition.

Bacteria growing in biofilms present a higher level of resistance to several antimicrobial agents compared to bacteria living in a planktonic state. For an oral antimicrobial agent to have meaningful in vivo efficacy, it is important that the active agent be capable of diffusing into the biofilm mass. The pen-
etration of a mouthrinse containing essential oils in dental plaque was tested in vivo using fluorescent dyes that differentiate live and dead bacteria. Thirteen minutes after a rinse with essential oils, 78.7% of bacteria in plaque samples were dead compared to 27.9% following rinsing with a placebo. The results clearly demonstrated that the essential oils were capable of penetrating the plaque mass and exert their antimicrobial effect on bacteria growing within biofilms. These findings were confirmed by additional studies that demonstrated in vivo antimicrobial effects of mouthrinses containing essential oils on preexisting plaque. Forty-four days of twice-daily rinsing with an essential oils-containing mouthrinse resulted in statistically significant reductions compared to a placebo mouthrinse in supragingival levels of Veillonella sp. (52.3%), Capnocytophaga sp. (74.1%), Fusobacterium nucleatum (81.5%) and total anaerobes (88.5%). Moreover, this regimen also resulted in statistically significant reductions in subgingival plaque samples of target microorganisms ranging from 54.1 to 69.1%, suggesting that rinsing with essential oils could also impact the subgingival microbiota. Using a similar design, the same group reported on the impact that rinsing for 14 days with an essential oil mouthrinse had on the subgingival levels of Porphyromonas gingivalis, Veillonella sp., F. nucleatum and total anaerobes. Thirty seven subjects with a minimum of 4 sites with probing depth of 4 to 7 mm were enrolled in the study. The results demonstrated statistically significant reductions in all target microorganisms in the essential oils group compared with the placebo group: P. gingivalis (64.5%), Veillonella sp. (56.6%), F. nucleatum (76.6%) and total anaerobes (74.9%). The authors suggested that the subgingival antimicrobial effect of the mouthrinse was mediated primarily by disruption of the contiguous supragingival biofilm. In addition, a regimen of twice daily rinsing with essential oils for 14 days can reduce the levels of total anaerobes, Gram-negative anaerobes and volatile sulphur compound-producing organisms in plaque and on the dorsum of the tongue up to 12 hours after the final rinse, indicating a certain level of substantivity.

Several studies with a minimum of 6 months of follow-up using a double-blind, controlled, randomized design have reported on the adjunctive effects of essential oil mouthrinses on decreasing plaque accumulation and development of gingivitis. These reports have described an average reduction of 32.9% in plaque accumulation (QHI), and of 22.1% in gingivitis (MGI) (Table 1) in the groups using essential oils. However, measures of total plaque reductions do not address the effects of these agents on specific areas of the oral cavity more prone to plaque accumulation and gingivitis such as the interproximal surfaces of posterior teeth. Some of the studies listed in Table 1 have addressed the efficacy of essential oils-containing mouthrinses in reducing plaque and gingivitis in interproximal areas. One study compared brushing plus the use of an essential oil mouthrinse (BEO) with brushing and flossing (BF) and brushing and rinsing with a placebo mouthrinse (B). After six months, the BEO and BF groups had statistically significantly lower mean interproximal MGI compared to the B group. The authors concluded that rinsing twice daily with an essential oils-containing mouthrinse was as efficient as daily flossing in reducing interproximal plaque and gingivitis. Another study examined the adjunctive benefit of essential oils in reducing plaque and gingivitis in subjects who brush and floss regularly. After six months the group that in addition to brushing and flossing used the essential oil mouthrinse had statistically and clinically significantly lower mean MGI and PI (Sillness-Löe Plaque Index) on the interproximal surfaces of posterior teeth. Some of the studies listed in Table 1 have addressed the efficacy of essential oils-containing mouthrinses in reducing plaque and gingivitis in interproximal areas.

### Table 1 - Antiplaque and antigingivitis effects of essential oil mouthrinses.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>N</th>
<th>Duration</th>
<th>*Plaque QHI</th>
<th>*Gingivitis MGI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gordon et al.</td>
<td>1985</td>
<td>85</td>
<td>9 months</td>
<td>19.5%</td>
<td>23.9%</td>
</tr>
<tr>
<td>DePaola et al.</td>
<td>1989</td>
<td>107</td>
<td>6 months</td>
<td>34.4%</td>
<td>33.7%</td>
</tr>
<tr>
<td>Overholser et al.</td>
<td>1990</td>
<td>124</td>
<td>6 months</td>
<td>36.1%</td>
<td>35.9%</td>
</tr>
<tr>
<td>Charles et al.</td>
<td>2001</td>
<td>316</td>
<td>6 months</td>
<td>56.1%</td>
<td>22.9%</td>
</tr>
<tr>
<td>Sharma et al.</td>
<td>2002</td>
<td>301</td>
<td>6 months</td>
<td>37.5%</td>
<td>7.9%</td>
</tr>
<tr>
<td>Bauroth et al.</td>
<td>2003</td>
<td>314</td>
<td>6 months</td>
<td>20.0%</td>
<td>11.1%</td>
</tr>
<tr>
<td>Sharma et al.</td>
<td>2004</td>
<td>237</td>
<td>6 months</td>
<td>51.9%</td>
<td>21.0%</td>
</tr>
</tbody>
</table>

*Reduction in the Index. QHI - Turesky modified Quigley-Hein index. MGI - Modified Gingival Index.
Index) than individuals in the placebo group (21.0 and 51.9%, respectively), suggesting that essential oils could be an effective adjunct to regular brushing and flossing.

In these studies, visual assessment of plaque removal and gingivitis reduction was performed to determine antiplaque and antigingivitis effects in interproximal areas. Due to the wide area of interproximal surfaces of premolars and molars, it is difficult to visually determine if plaque was completely removed from the interproximal area. Likewise, gingivitis might have been reduced in the vestibular and lingual/palatal portion of the interdental papilla but not in the col area. In order to demonstrate the antimicrobial efficacy of essential oil mouthrinses in the interproximal area, the effect of 11 days of twice daily rinse with an essential oils-containing mouthrinse on the interproximal levels of *Streptococcus mutans* was assessed. The results demonstrated a reduction of 75.4% in total recoverable *S. mutans* from interproximal spaces in the essential oils group. Another study evaluating the effects of essential oils in the total viable bacteria in the interproximal spaces demonstrated a statistically significant reduction of 43.8% in interproximal bacteria 5 minutes after a single 30-second rinse.

**Effects of Triclosan/copolymer dentifrices on periodontal biofilms and gingivitis**

Dentifrices containing 0.3% triclosan and 2% polyvinyl methyl ether maleic acid copolymer (copolymer) have demonstrated significant antiplaque and antigingivitis properties. Triclosan is a broad-spectrum antimicrobial agent, which acts on the bacterial cytoplasmic membrane. It prevents essential amino acid uptake at bacteriostatic concentrations, while at bactericidal concentrations it causes disorganization of the bacterial membrane leading to leakage of cellular contents. The copolymer acts by increasing the retention of triclosan on tooth and oral soft tissue surfaces. A greater uptake of triclosan to plaque and saliva was observed when copolymer was added to triclosan-containing dentifrices. Tests of the substantivity of toothpaste slurries demonstrated that dentifrices containing triclosan/copolymer reduced the proportions of vital bacteria in plaque for up to 24 hours. The effects of one-week, twice-daily use of triclosan/copolymer dentifrice on the levels of *Veillonella* sp., *Fusobacteria* sp., total anaerobes and hydrogen sulfide-producing bacteria were tested on samples of plaque, saliva and tongue scrapings 6 and 12 hours after the final brushing. Significant reductions (88 to 96%) in total anaerobes were reported for the triclosan/copolymer group in the 3 sites at both time points compared with the control group. In plaque samples, *Fusobacteria* sp. decreased by 77 and 92% and *Veillonella* sp. decreased by 90 and 85% in the triclosan/copolymer group versus the control group 6 and 12 hours after brushing, respectively.

The effectiveness of dentifrices containing triclosan/copolymer in reducing plaque accumulation and gingivitis was examined in a systematic review. Only randomized clinical trials with at least 6 months of follow-up were included. A meta-analysis was performed on the results of 16 studies involving 1,150 control and 1,176 test subjects. The results demonstrated that the triclosan/copolymer toothpaste was capable of reducing plaque (23% using the QHI and 49% using the plaque severity index) and gingivitis (23% using the GI and 49% using the gingivitis severity index). Table 2 presents the percentage reduction in plaque and gingivitis obtained in several studies examining the effectiveness of a triclosan/copolymer dentifrice. Most studies demonstrated statistically significant improvements in both parameters after 6 months of use of the product. In a few studies, however, differences between control and test groups were only significant at 3 months but not at 6 months (Table 3). After six months, the experimental group had a 34.9% reduction in the levels of preexisting plaque and gingivitis has also been examined. After six months, the experimental group had a 34.9% reduction in the levels of preexisting plaque and a 25.7% reduction in gingivitis, compared with the placebo group.

In a 3-year study examining the clinical and microbiological changes associated with the combined use of a powered toothbrush and a triclosan/copolymer dentifrice, the authors could not demonstrate any clinical benefit of the use of the antimicrobial...
toothpaste over the use of a manual toothbrush and a fluoride-containing dentifrice. Microbiological samples were collected at baseline and after 1, 2 and 3 years and individually analyzed using the checkerboard DNA-DNA hybridization technique. Both groups resulted in similar reductions in mean total DNA probe count ($\times 10^5$). The levels of the 40 species tested were reduced by both preventive regimens to similar levels. In this study both groups were part of comprehensive prevention programs involving recall visits every 6 months for reinforcement of homecare procedures and professional cleaning. In addition, subjects were provided with the appropriate toothbrush, dentifrice and interdental devices on every visit. A possible interpretation of the failure of this study in demonstrating an adjunctive antiplaque and antigingivitis effect of the triclosan/copolymer toothpaste is that when meticulous mechanical plaque control is performed, any additional benefit of an antimicrobial agent is minimal.

### Effects of Triclosan/copolymer dentifrices on periodontitis progression

A 3-year double-blind, controlled, randomized trial examined the effects of twice daily use of triclosan/copolymer toothpaste on the prevention of attachment loss in adolescents (11-13 years old). The study demonstrated that for individuals with high mean pocket depths at baseline (2.92 - 4.19 mm), the use of triclosan/copolymer resulted in lower mean attachment loss at the end of the study. The effects of triclosan/copolymer in the prevention of periodontitis in maintenance patients have also been examined. In one study the use of triclosan/copolymer dentifrice was capable of slow-
ing the progression of periodontitis in maintenance subjects during the 3 years of the trial.\textsuperscript{55} The daily use of triclosan/copolymer toothpaste resulted in a lower frequency of deep periodontal pockets and a lower number of sites with additional attachment and bone loss by the end of the study. Conversely, a trial examining the 3-year effect of the combined use of powered toothbrush and triclosan dentifrice in periodontal maintenance subjects failed to demonstrated superior clinical and microbial outcomes for the test group compared with a control group using manual toothbrush and a standard fluoride toothpaste.\textsuperscript{56} The discrepancy in the outcome of the two studies can be justified by differences in study design. In the former study no professional subgingival therapy was provided, while in the latter subgingival debridement was performed every 6 months. It seems that when meticulous subgingival mechanical debridement is performed, the benefits of the use of antimicrobial toothpaste cannot be demonstrated. In the absence of such supportive maintenance therapy, it is possible that the use of the triclosan/copolymer dentifrice resulted in a reduction in the levels of subgingival species, leading to additional clinical benefits. In fact, it has been demonstrated that careful supragingival plaque control with the adjunct use of triclosan/copolymer toothpaste can affect the subgingival microbiota.\textsuperscript{57}

Safety of oral antimicrobial agents

The prolonged use of antimicrobial agents as adjuncts to mechanical plaque control raises two main concerns regarding safety: the development of resistant microorganisms and the risk of oral cancer associated with the alcohol content of mouthrinse formulations. Oral antiseptics affect a broad spectrum of microorganisms and have non-specific antimicrobial mechanisms, which affect a number of different targets in the microbial cell, inhibiting a variety of cellular processes.\textsuperscript{58} This is probably the main reason for the lack of development of bacterial resistance to antiseptics. Point mutations in the microorganism might affect one of the mechanisms of action of a given antiseptic, but rarely would result in resistance to all mechanisms. In fact, several studies have examined the long-term microbiological safety of dentifrices and mouthrinses containing antiplaque agents and none of them have reported the development of opportunistic infections, overgrowth of pathogenic bacteria or the development of resistant species.\textsuperscript{58}

Concerns about the high content of alcohol in certain mouthwash formulations are justified based on the well established association between alcohol consumption and an elevated risk to develop oropharyngeal cancer. However, a review of studies that suggested a correlation between mouthrinses containing alcohol and oral cancer by the American Dental Association (ADA) and by the Food and Drug Administration (FDA) concluded that these studies presented several deficiencies.\textsuperscript{59} Among the problems detected in the studies there were the non-exclusion of subjects due to the consumption of alcoholic beverages or smoking and the inclusion of pharyngeal cancer in the analysis, while oral exposure to mouthrinses should not affect the pharynx. Two recent reviews on the topic concluded that there was no support in the literature for an association between mouthrinses containing alcohol and oropharyngeal cancer.\textsuperscript{60,61}

Concluding remarks

- The adjunct use of essential oil mouthrinses and triclosan/copolymer dentifrices can result in additional reductions in plaque and gingivitis, particularly in hard-to-reach areas such as interproximal spaces.
- The use of chemical agents might help reduce the accumulation of biofilms on soft tissue surfaces of the oral cavity, potentially delaying plaque accumulation on teeth.
- Antimicrobial agents such as essential oils are capable of affecting bacteria growing in supragingival biofilms and disrupt preexisting plaque.
- The use of essential oil mouthrinses and dentifrices containing triclosan/copolymer might affect the subgingival microbiota through the disruption of the contiguous supragingival plaque.
- The use of a dentifrice containing triclosan/copolymer might prevent the progression of attachment loss in adolescent with a high risk of devel-
opining “early periodontitis”.

- The use of a dentifrice containing triclosan/copolymer might prevent further loss of attachment in patients with a history of periodontitis, particularly in the absence of a supportive periodontal therapy that includes subgingival debridement.
- Despite its limitations, meticulous mechanical supragingival plaque control is capable of reducing plaque accumulation and preventing gingivitis even without the adjunct use of antimicrobials.
- Well executed supra and subgingival mechanical plaque control is capable of preventing further loss of attachment in subjects under periodontal maintenance even without the adjunct use of antimicrobials.
- The long term use of oral antimicrobials does not seem to lead to undesirable microbiological side effects such as the development of resistant species.
- The use of mouthrinses containing alcohol does not seem to increase the risk of oral cancer.

References

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