Commercial antimicrobials mouthrinses on caries and periodontitis-related biofilm control: a review of literature

Enxaguatórios comerciais antimicrobianos sobre o controle do biofilme relacionado à cárie dentária e à doença periodontal– uma revisão de literatura

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ABSTRACT

This review aims to discuss the antimicrobial potential of different mouthrinses in respect to the control of dental caries and periodontal disease. The survey was conducted using PubMed and the following keywords: “antimicrobial agent” or “antiplaque agent”, “dental biofilm” and “dental caries” or “periodontal disease” or “gingivitis”. Only studies published in English, from 2011 to 2015, in journals with impact factor greater than 0.8, were selected. We found a total of 22 papers, 13 related to dental caries and 9 related to periodontal disease. Among the 13 studies involving cariogenic bacteria and/or biofilm, 6 were conducted in vitro, 3 in situ and 4 in vivo. Among 9 studies involving periodontal disease, 2 were in vitro and 7 in vivo. The main active agents tested were: CHX-Chlorhexidine, CPC-cetylpyridinium chloride and EO-Essential oils (alcohol/or alcohol-free). CHX was compared to EO in 6 studies, showing superiority in 3 studies, similarity in 1 study and inferiority in 2 studies. CPC has shown lower effect in plaque reduction compared to CHX and EO. There is still controversy about the effect of alcohol, but some studies have shown superiority for EO and CHX with alcohol on cariogenic and periodontopathogenic biofilms, respectively, when compared to alcohol-free version; for CPC, no difference was found. More clinical studies are needed for better understanding the mechanism of action and the differences in performance among the antiplaque agents.

KEYWORDS

Antimicrobial agents; Dental biofilm; Dental caries; Oral diseases; Periodontitis.
**INTRODUCTION**

The oral cavity is directly in contact with microorganisms [1]. Saliva, gingival fluid and our diet supply nutrients for them, making the environment propitious to microbiota development [2,3]. Microbiota can be organized as biofilm that potentially can cause oral diseases as dental caries and periodontitis [4].

Dental caries is one of the most relevant oral chronic diseases caused by microorganisms from different species organized in a supragingival biofilm. The cariogenic microorganisms metabolize sugar, especially sucrose derived from the diet, producing acids that reduce the biofilm pH and cause tooth decay [4,5]. The main cariogenic microorganisms present in biofilm are *S. mutans*, *Lactobacillus*, bifidobacteria and fungi. *S. mutans*, in particular, produce insoluble extracellular polysaccharides from sucrose in the biofilm matrix, increasing metabolic efficiency and protecting themselves against host defenses mechanisms [4,6].

On the other hand, subgingival biofilm rich in anaerobic and gram-negative bacteria (*A. actinomyctecomitans*, *T. forsythia*, *Campylobacter spp.*, *Capnocytophaga spp.*, *E. corrodens*, *F. nucleatum*, *P. gingivalis*, *P. intermedia*) is related to periodontal disease, a common cause of tooth loss in adults [7].

The mechanical disorganization of dental biofilm by toothbrushing is extremely important to prevent dental caries [8] and gingivitis [9], but sometimes insufficient for patients who have unfavorable conditions as, for example, xerostomia [10] and using fixed orthodontic appliances. The use of antimicrobials agents may be an alternative for those patients at high risk of dental caries [10] and periodontal disease [11,12].

Among the active agents, chlorhexidine digluconate (CHX), Cetylpyridinium Chloride (CPC) and essential oils (EO) [13] are the most used by the population, who applied them for halitosis control [14]. CHX is considered a gold-standard antimicrobial agent applied in patients with periodontal diseases. It has been indicated as a temporary coadjuvant to regular oral hygiene procedures, as a preoperative and/or postoperative rinse either [15].

Despite the popularity of the antimicrobial agents often found in supermarkets, there is sparse information about their efficacy on the control of oral diseases. Therefore, the aim of this review was to compile information about the efficacy of the main commercial antimicrobial agents applied to prevent tooth decay and periodontal disease.

**REVIEW OF LITERATURE**

The survey was conducted using PubMed and the following keywords: “antimicrobial agent” or “antiplaque agent”, “dental biofilm” and “dental caries” or “periodontal disease” or “gingivitis”. Only studies published in English, from 2011 to 2015, in journals with impact factor greater than 0.8, were selected. We found a total of 22 papers, 13 related to dental caries and 9 related to periodontal disease, involving *in vitro*, *in situ* and *in vivo* models.

**Commercial Agents and Biofilm/Dental Caries**

Chlorhexidine (CHX) is considered a gold standard antimicrobial agent applied in dentistry. Accordingly, most studies testing new antimicrobial agents have included CHX as a positive control. Cetylpyridinium chloride (CPC), also a cationic agent as chlorhexidine, is indicated to combat dental plaque and halitosis [16]. Essential oil (EO: eucalyptol, thymol, salicylate and menthol), a non-ionic agent, is other agent popularly applied to control dental plaque [17]. The agents are mostly available as mouthrinses.

Different commercial mouthrinses containing CHX, in concentrations ranged from 0.05 to 0.2%, were compared to EO (formulae with alcohol) and water (negative control) using multispecies biofilms (*A. naeslundii*, *V.
dispar, F. nucleatum, S. mutans, S. oralis and C. albicans). The total CFU were determined using Columbia blood agar, and the S. mutans and S. oralis CFUs were counted using Mitis-Salivarius agar. The treatments were done after 16.5, 24.5, 40.5, and 48.5 h of biofilm formation. After a total time of 64.5 h, CFUs were determined for total microorganisms (A. naeslundii, V. dispar, F. nucleatum, S. mutans, S. oralis and C. albicans), S. mutans and S. oralis. The total CFU numbers were not significant different among the mouthrinses. Biofilm formation was reduced in 7 log10 steps by 0.2% CHX (formulae with alcohol), and in 3 log10 steps by EO, 0.05% CHX (formulae with alcohol), and 0.12 and 0.2% CHX (without alcohol) solutions compared to water [18].

The effect of three commercial mouthrinses (1- 0.12% CHX plus 0.05% CPC; 2- 0.12% CHX; 3- 0.2% zinc chloride with 1.5% hydrogen peroxide-Zn), on bacteria adherence (S. mutans, S. faecalis, S. gordonii, A. viscosus and Mixed culture) to hydroxyapatite surfaces was evaluated. The % of viable bacteria adhered to hydroxyapatite ranged from 8-19% for CHX plus CPC; 11-17% for CHX and 79-89% for Zn. Therefore, both CHX and CHX plus CPC were effective against the tested bacteria, while Zn was not [19].

Wakamatsu et al. [20] compared the penetration kinetics of four mouthrinses (CHX, EO, CPC and isopropylmethylphenol- IPMP) into S. mutans biofilm. The penetration velocities were determined by monitoring fluorescence loss between 30 s and 5 min exposure. EO showed the best penetration, but within 30 s no mouthrinse had any antiplaque effect. After 30 s, EO induced the highest reduction in CFU number, but not in bacteria detachment compared to the other mouthrinses.

Nanoemulsions prepared with 25% soybean oil, 1% CPC and 10% Triton X-100-TRI (NE) were applied on S. mutans, L. casei, C. albicans and A. viscosus both isolated and in a mixed-culture. S. mutans and L. casei biofilms were stained using a live/dead kit. MIC and MBC were determined for all microorganisms isolated and in a mixed-culture. The time kinetics was also analyzed for all microorganisms (1, 5, 15, 30 and 60 min) using optical density. The adherence of microorganism on glass plates (24 h) and the growth of biofilm (72 h) were analyzed after fixing and staining, using optical density. NE has shown reduced in 83% the viability of S. mutans and L. casei biofilm compared to negative control. MIC and MBC of NE were 9- to 27-fold smaller than those from CHX (positive control). In respect to killing curves, NE had a faster and powerful effect compared to CHX. The level of adhesion on glass surface was reduced by 94.2 to 99.5% in NE treated groups compared to positive (CHX) and negative controls. The anti-adherence and anti-biofilm effects of NE suggest a promising anti-caries action [21].

Commercial rinses containing 0.05% CPC, alcohol or free-alcohol, were compared with 0.05% fluoride mouthwash (F) and 0.12% chlorhexidine (positive control-CHX). MIC was firstly determined for each mouthrinse considering 25 microorganism species associated with oral diseases. The second part of the study evaluated the antimicrobial activity using supragingival biofilm collected from 15 subjects, which was exposed ex vivo to the mouthrinses for 5-7 days in anaerobic environment. MIC values were significantly lower for both CPC rinses compared to fluoride rinse especially against gram-negative bacteria (most involved in halitosis etiology), showing a broad-spectrum activity. CHX had the greatest antimicrobial effect. This ex vivo model showed no difference between CPC rinses formulated with alcohol or without alcohol. Both CPC (> 90% killing) and CHX (98% killing) showed higher antimicrobial activity compared to F [22].

The effect of antimicrobial mouthrinses was also studied in adult patients under orthodontic treatment. The patients were treated with 0.1% CHX alcohol-free, essential oil (alcohol/alcohol-free) or negative control.
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(1% hydroalcoholic solution) for 4 days (1x30s/day). Supragingival biofilm and microorganism on tongue were collected and analyzed for UFC counting (S. mutans). All mouthrinses were similarly able to significantly reduce the number of S. mutans colonies compared to control for both samples (tongue and biofilm) [23].

The antiplaque effect of EO with or without alcohol was compared in vivo. Thirty subjects were divided into two groups (EO with and without alcohol). They rinsed twice a day for 3 days. EO with alcohol showed better plaque inhibitory effect (plaque index of 2.18 in whole mouth) than alcohol-free solution (plaque index of 2.46) [24].

Oyanagi [25] compared 0.05% CHX, 0.2% benzethonium chloride, EO (0.9% 1.8-cineol, 0.06% thymol, 0.05% Methyl salicylate, 0.04% l-Menthol and 27% Ethanol), 7% povidone iodine (PVP-I) and PBS (negative control) using planktonic cariogenic bacteria (S. mutans/ S. sobrinus) and biofilm models. Additionally, two mouthrinses (CHX and EO) were evaluated using biofilm-induced caries and a secondary caries model. EO and PVP-I were the best treatments in reducing the cells viability and CFU counts in planktonic culture and biofilm (especially in top and middle layer). EO further had the best inhibitory effect on the progression of demineralization, showing potential to prevent dental caries.

Albertsson et al. [26] evaluated the antimicrobial effect of EO and CHX alcohol-free mouthrinses on S. mutans and Lactobacillus in saliva. Twenty healthy volunteers applied the mouthrinses twice times during 16 days after the regular mechanical oral hygiene. Saliva was collected and analyzed for CFU/ml. Only CHX rinse showed a significant reduction in S. mutans and Lactobacillus counting, while EO did not have antimicrobial effect.

Two mouthrinses, EO (0.092% of eucalyptol, 0.042% menthol, 0.060% of methyl salicylate and 0.064% of thymol) and 0.2% CHX, were tested on biofilm in situ. Bacterial viability, thickness and covering grade were evaluated after 4 days of applying each of the mouthrinses (2 times x 30s/day). CHX showed 13.2% and EO 14.7% of live bacteria. CHX was better in reducing biofilm thickness compared to EO (CHX 6.5 μm vs. EO 10.0 μm) and covering grade (CHX 20.0% vs. EO 54.3%). CHX showed better antiplaque effect compared to EO [27].

The effect of CPC (concentrations of 0, 0.025%, 0.05%, 0.075%, and 0.1%), applied twice day for 1 min, during early (0h to 50h) and mature (48h to 98h) S. mutans biofilm formation, was determined. All CPC concentrations showed complete anti-biofilm activity during early biofilm formation. For old biofilm, the highest CPC concentrations had effect on dry weight, viability and acidogenicity, but they had no effect on water-insoluble extracellular polysaccharides production. Therefore, CPC has inhibitory effect on young S. mutans biofilm only [28].

Hannig et al. [29] compared the effect of fluoride solution (100 ppm F as AmF and 150 ppm F as NaF) to 0.2% CHX (positive control) on biofilm adherence to enamel and dentin in situ after 8 h of 1 min-rinse. The bacterial viability and CFU for total microorganism were determined. In the control group, significantly higher amounts of adherent bacteria were detected on dentin (4.8 x 10^6 ± 5.4 x 10^6 bacteria/cm^2) than on enamel (1.2 x 10^6 ± 1.5 x 10^6 bacteria/cm^2). Chlorhexidine significantly reduced the amount of adherent bacteria (dentin: 2.8 x 10^5 ± 3.4 x 10^5 bacteria / cm^2; enamel: 4.2 x10^5 ± 8.7 x 10^5 bacteria/cm^2). Rinses with the fluoride solution also significantly reduced bacterial adherence to dentin (8.1 x 10^5 ± 1.5 x 10^6 bacteria/cm^2). The viability was reduced by both chlorhexidine and fluoride. While a significant reduction of bacterial adherence on enamel and dentin was seen for chlorhexidine, F reduced the bacterial adherence on dentin only.

Rabe et al. [30] compared the antimicrobial effect of 0.1% CHX with 0.2% NaF. Enamel discs were mounted on healing abutments in the premolar region of three subjects for 7 days. After
this period, the treatment was done for 1 min. Then, the architecture, bacterial viability and total biomass of the biofilm were evaluated using fluorescence methods. The biofilm architecture was similar for both groups, however CHX had effect on the biofilm surface, while F caused cell damage in the middle and deep biofilm layers. Both rinses were able to significantly reduce the bacterial vitality (63% vs. 95% in control) and the total biomass (6.5 x 10^6 arbitrary units/mm^2 for control, 0.82 x 10^6 arbitrary units/mm^2 for CHX and 0.87 x 10^6 arbitrary units/mm^2 for F). Rinse with CHX has antimicrobial effect in the cell/liquid interface at the top of biofilm. NaF, however, is able to penetrate and exert effect in the middle and deep levels of the biofilm.

Some in vitro studies have shown that fluoride reduces the production of lactate and the biomass of S. mutans biofilm when applied in high concentrations [31,32], however, other studies have shown no differences in lactate production, CFU number and pH drop by the application of fluoride compared to control in S. mutans biofilm [33,34]. The antimicrobial effect of fluoride is still not a consensus.

Table I summarizes the results found in the above-cited studies. Generally, CHX and EO seem to be the best antimicrobial agents against cariogenic bacteria. Both CHX and EO showed better antimicrobial effect than CPC, while F has limited antimicrobial effect. Few studies have analyzed the impact of these mouthrinses on the prevention of tooth demineralization, which should be the most relevant question to be answered.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Active component</th>
<th>Type of microorganism and model/treatment</th>
<th>Response variable</th>
<th>Results</th>
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<tbody>
<tr>
<td>Guggenheim &amp; Meier</td>
<td>1. 0.1% CHX (alcohol)</td>
<td>Multispecies biofilm/ treatment of 1 min each 12 h, during 845 h in vitro</td>
<td>Total microorganisms (A. naeslundii, V. dispar, F. nucleatum, S. mutans, S. oralis and C. albicans, S. mutans and S. oralis CFU numbers.</td>
<td>The total CFU numbers did not differ among the mouthrinses. 0.12% CHX (alcohol-free), 0.2% CHX (alcohol), 0.05% CHX (alcohol) and Listerine reduced the number of CFU of S. mutans in 7 log_{10} steps compared to control (water). 0.1% CHX (alcohol), 0.2% CHX (alcohol), 0.15% CHX (alcohol) reduced the number of CFU of S. oralis in 3 log_{10} steps compared to control (water).</td>
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<td>Marchetti et al.</td>
<td>1. EO (alcohol-free) 2. EO (alcohol)</td>
<td>Cariogenic biofilm/ treatment twice a day during 3 days in vivo</td>
<td>Plaque index-PI in whole mouth</td>
<td>EO (alcohol) showed better inhibitory effect compared EO (alcohol-free)</td>
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<td>Sreenivasan et al.</td>
<td>1. 0.05% CPC+ (alcohol) 2. 0.05% CPC- (alcohol-free) 3. 0.05% Sodium Fluoride (alcohol) 4. 0.12% CHX (alcohol)</td>
<td>(A) 25 Species associated with dental caries in planktonic phase. (B) Supragingival biofilm collected from 15 subjects and treated with agar media containing 1% of each mouthrinse during 5–7 days</td>
<td>MIC and CFU of S. gordonii, S. mutans, C. albicans, A. meyeri, A. viscosus.</td>
<td>The cariogenic bacteria were inhibited by &lt;6% CPC+ solution, &lt;3% CPC- solution and &lt;50% F solution. Both CPCs had similar effect and were better than F. For CPC, CPC mouthrinses (&gt;90% killing) and CHX rinse (&gt;98% killing) were better compared to F.</td>
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<td>Ramalingam et al.</td>
<td>1. 1% CPC and 10% Triton X-100-TRI (NE) 2. 0.02% CHX (alcohol-free)</td>
<td>S. mutans, L. casei, A. viscosus, C. albicans and mixed culture biofilm (72 h)</td>
<td>Level of microorganisms adhesion on glass surface and MIC/ MBC values</td>
<td>MIC and MBC of NE were 9- to 27- fold smaller than those from CHX (positive control). NE has shown reduced in 83% the viability of S. mutans and L. casei biofilm compared to negative control. The level of adhesion on glass surface was reduced by 94.2 to 99.5% in NE treated groups compared to positive (CHX) and negative controls, respectively.</td>
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| Oyanagi et al. [25]      | 1. 0.05% CHX (alcohol-free)  
2. 0.2% benzethonium chloride  
4. 7% povidone iodine (PVP-I) | (A) S. mutans and S. sobrinus in planktonic phase (48-hour incubation).  
(B) Multispecies Biofilm (S. mutans, S. sobrinus and S. gordonii)/ treatment (1x60s) for 7 days in vitro | CFU counting of cariogenic bacteria and enamel lesions analysis.                   | EO and PVP-I killed more planktonic cariogenic bacteria and bacteria embedded in biofilms compared to PBS, CHX and benzethonium.  
EO presented the smallest lesions among the three groups (PBS, CHX and PVP-I)                                                          |
| Albertsson et al. [26]   | 1. 0.1% CHX (alcohol-free)  
3. CPC (alcohol-free)  
4. 0.025% CPC (alcohol-free)  
5. Negative control (water) | Inhibition on S. mutans and Lactobacillus in saliva/ treatment twice a day for 16 days in vivo.            | CFU counting                                                                      | CHX (alcohol-free) showed significant reduction in S. mutans and Lactobacillus, while the EO rinse did not. |
| Hanning et al. [29]      | 1. 0.2% CHX (alcohol-free)  
2. 100 ppm AmF and 150 ppm NaF  
3. 0.1% CHX (alcohol-free) | Biofilm formation (8h) on enamel and dentin/ treatment for 1 min in situ.                              | Viability and Biofilm adherence                                                    | The viability was reduced by both solutions, while CHX was the only one showing inhibition of bacterial adherence on both enamel and dentin. |
| Ulkur et al. [23]        | 1. 0.1% CHX (alcohol-free)  
2. EO (alcohol-free)  
3. 0.075% CPC (alcohol-free)  
5. Negative control (alcohol-free) | Treatment for 4 days in vivo. Groups 1 and 3 rinsed 2x30s/ day, while Group 2 rinsed 3x30s/day.          | CFUs counting for S. mutans on the teeth and tongue surfaces                       | All mouthrinses were similarly able to significantly reduce the number of S. mutans.                                                     |
| Babu and Garcia-Godoy [19]| 1. 0.02% CHX plus 0.05% CPC (alcohol-free)  
2. 0.02% CHX (alcohol-free)  
3. 0.2% zinc chloride plus 15% hydrogen peroxide (alcohol-free) | S. mutans, S. faecalis, S.gordonii/A. viscosus in a mixed culture biofilm (48h)/ treatment during 1 min in vitro | Bacterial adhesion, viability and CFU counts                                      | Both CHX and CHX plus CPC were effective against the tested bacteria in all assay, while Zn was not.                                   |
| Wakamatsu et al. [20]    | 1. 0.12% CHX (alcohol-free)  
3. CPC (alcohol-free)  
4. Isopropl methyl phenol (alcohol-free) | S. mutans biofilm/ treatment during 30s in vitro                                                      | Penetration velocities and antimicrobial effect by monitoring fluorescence loss of calcine AM-stained biofilms with time-lapse confocal laser scanning microscopy | EO showed the best penetration in biofilm. None of the mouthrinses have antimicrobial effect on S. mutans.                                |
| Pandit et al. [28]       | 1. 0.025% CPC  
2. 0.05% CPC  
3. 0.075% CPC  
5. Negative control (water) | S. mutans biofilm/ treatment each 12h, during 98 h in vitro                                           | Weight, viability and acidogenicity on Early (0h to 50h) and mature (48h to 96h) S. mutans biofilm. | In early biofilm all concentrations had antimicrobial effect. In mature biofilm, only the highest concentrations of CPC had effect on dry weight, viability and acidogenicity. |
| Quintas et al. [27]      | 1. EO (alcohol)  
2. 0.2% CHX (alcohol) | Antiplaque effect in situ/ treatment twice a day during 4 days                                          | Bacterial viability, thickness and covering grade of dental plaque                | CHX and EO presented 13.2% and 14.7% of live bacteria (ns). CHX was more efficient in reducing plaque thickness and covering grade compared to EO. |
| Rabe et al. [30]         | 1. 0.3% CHX (alcohol-free)  
2. 0.2% NaF (alcohol-free) | Cariogenic biofilm in situ/ treatment for 1 min a day during 7 days                                   | Architecture, bacterial viability and total biofilm biomass                       | CHX and NaF caused a similar effect.                                                                                                     |

Abbreviations: CHX – chlorhexidine; CFU – colony forming units; EO – essential oil; PI – plaque index; CPC - cetylpyridinium chloride; MIC – minimum inhibitory concentration; MBC - minimum bactericidal concentration; NE – Nanoemulsion; PVP-I – Povidone iodine; PBS – Phosphate Buffered Saline; ppm – parts per million; Zn – Zinc chloride; PCA – Pyrrolidone Carboxylic Acid; AM – Acetoxymethyl; NaF – Sodium fluoride.
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CHX, as previously shown, has been widely tested as antiplaque agent. In this in vivo study, the authors tested two formulations. The volunteers rinsed twice a day 0.12% CHX (alcohol) or 0.1% CHX (alcohol-free) with 0.1% of Formaldehyde (CHX-F) during 7 days. After the treatment, plaque indexes were recorded. The mean plaque of first group (0.76±0.38) was significantly lower compared to the second group (1.43±0.56), showing that alcohol might have some influence on the antiplaque effect of CHX [35].

A crossover study was done with ten volunteers using an experimental gingivitis model. The volunteers applied 0.2% chlorhexidine mouthrinses with alcohol (CHXA) or alcohol-free (CHXNA) for 21 days (2x1min/day). The plaque index (PI), gingival inflammation (GI) and discoloration teeth (DI) were evaluated. Both solutions presented similar PI (CHXA initial 0.55 ± 0.23 and final 0.69 ± 0.23 vs. CHXNA initial 0.52 ± 0.15 and final 0.75 ± 0.19), GI (CHXA initial 0.64±0.32 and final 0.73 ± 0.11 vs. CHXNA initial 0.61±0.24 and final 0.77 ± 0.33), and DI (CHX A initial 0.0±0.0 and final 0.20 ± 0.30 vs. CHXNA initial 0.0 ± 0.0 and final 0.06 ± 0.06). Therefore, both formulations presented comparable levels of action [36].

Three mouthrinses (0.12% chlorhexidine plus 0.05% CPC; 0.12% CHX pure and 0.12% CHX plus NaF) were tested for the inhibition of oral bacteria related with periodontal diseases (S. oralis, A. naeslundii, V. parvula, E. nucleatum, A. actinomyctemcomitans, P. gingivalis) and on biofilm formed in vitro. The effect of the mouthrinse was analyzed in bacteria under planktonic phase, which were treated for 1 min using the short interval-killing test. The antimicrobial effect was measured as CFU/mL and no difference was found among the rinses. For biofilm formation, the bacteria were grown on sterile ceramic calcium hydroxyapatite (HAP) discs for 12 h at 37 °C using a bioreactor. The discs were immersed in the mouthwash for 2 min and the biofilm cultivated for 5 days. The viable cells were analyzed using culture methods, scanning electron microscopy (SEM), Live/Dead staining and fluorescence in situ hybridization (CLSM). SEM showed a typical biofilm structure. The fluorescence in situ hybridization technique confirmed the presence of the six bacterial species in biofilms older than 3 days. The live/dead ratio revealed that the majority of cells were alive in 3-, 4- and 5-d biofilms. Cells in biofilms showed more tolerance compared with planktonic cells. In 4-d biofilm, CHX+CPC showed more antimicrobial effect than CHX+NaF and CHX [37].

Both CPC and EO were also compared in an in vivo study, in which 142 subjects wore the mouthrinses for 2 weeks (2x30s/day). The Modified Gingival Index (MGI), Plaque Index (PI) and bleeding Index (BI) were analyzed. EO demonstrated significant reduction in MGI (9.4%), PI (6.6%) and BI (29%) compared to CPC. EO presented clinical superiority compared to CPC in the short-term management of plaque and gingivitis [38].

Cortelli et al. [11] compared the antiplaque/antigingivitis effect of EO (0.092% eucalyptol, 0.042% menthol, 0.060% methyl salicylate and 0.064% thymol), 0.07% CPC and 5% hydroalcohol solution (control) in 354 healthy volunteers. They were instructed to rinse twice daily (30s each) for 6 consecutive months. The Modified Gingival Index (MGI), Plaque Index (PI) and bleeding Index (BI) were quantified at baseline, after 1, 3 and 6 months. After six months, EO (42.0%) and CPC (13.9%) demonstrated significant reduction in PI compared to negative control. EO (42.6%) and CPC (17.1%) also demonstrated significant reduction in MGI compared to negative control. EO (74.5%) and CPC (22.8%) demonstrated significant reduction in BI compared to negative control either. EO presented clinical superiority compared to CPC in the long-term management of plaque and gingivitis.
EO (with ZnCl₂ and NaF) was compared to 0.05% CPC (NaF) solutions. The subjects applied mouthrinses twice a day (30s) for 6 months. The PI and MGI were analyzed at baseline, after 3 and 6 months. EO mouthrinse showed significant superiority in reducing PI (23.6%) and MIG (19.5%) compared to CPC (4.2% and 1.7%) mouthrinses after 6 months. In respect to gingivitis, CPC was not different from control after 6 months. EO presented clinical superiority compared to CPC in the long-term management of plaque and gingivitis [39].

Sánchez et al. [40] evaluated the antimicrobial effect of three commercial mouthrinses (0.12% CHX plus 0.05% CPC, EO, and fluoride/stannous fluoride - AFSF) on mixed periodontopathogenic biofilm (S. oralis, V. parvula, A. naeslundii, F. nucleatum, A. actinomycetemcomitans and P. gingivalis) in vitro using ATP bioluminescence and CFU methods. 72h-biofilm was exposed to mouthrinses or control for 1 min. ATP bioluminescence showed antibacterial effect for all mouthrinses compared to PBS control. The lowest cell viability values were found for CHX plus CPC (1.38x10⁸ ± 8.54x10⁷ CFU/ml), followed by AFSF (1.42x10⁸ ± 9.03 x10⁷ CFU/ml), EO (1.67x10⁸ ± 1.17x10⁸ CFU/ml) and the negative control (2.55x10⁸ ± 1.63x10⁸ CFU/ml). Both CHX/CPC and F were similarly and more effective against biofilm compared to EO.

Commercial mouthrinse with 0.075% CPC (fluoride-free/alcohol-free) was compared with EO (fluoride-free/alcohol) in respect to antiplaque and antigingivitis effects in vivo. Fluoride-free/alcohol-free mouthwash was used as negative control. After 6 weeks, the subjects from CPC, EO and NC groups exhibited reductions in GI of 28.6%, 22.6% and 1.70%, respectively; while PI was reduced in 31%, 28% and 1.4% for CPC, EO and NC, respectively. Both mouthrinses provide a significant reduction in dental plaque and gingivitis [41].

A new rinse with CPC (alcohol-free) was tested to control plaque and gingivitis in 67 adults with moderate gingivitis during 6 months (3x30 s/day). PI, bleeding on marginal probing (BOMP) and stain (S) indexes were applied. The presence of A. actinomycetemcomitans, P. gingivalis, P. intermedia/nigrescens, T. forsythia, P. micra, Capnocytophaga spp., E. corrodens, Eubacterium spp. and F. nucleatum were determined in the biofilm. Significant reduction of the clinical parameters was observed for the tested CPC solution compared to placebo. Among the periodontopathogenic bacteria, P. intermedia showed a clear reduction after 3 and 6 months of CPC treatment. CPC shows ability to reduce biofilm accumulation after 3 and 6 months of use [12]

Based mainly on clinical trials as shown in Table II, EO has a superior antiplaque and antigingivitis effects compared to CPC, while EO has a similar efficacy compared to CHX. On the other hand, CHX has often been responsible for inducing undesired effects as tooth discoloration.
Table II - Effect of commercial antimicrobial mouthrinses on periodontitis/gingivitis or bacteria perio-related.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Active component</th>
<th>Type of microorganism and model/treatment</th>
<th>Response variable</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charles et al.</td>
<td>1. EO (alcohol) 2. 0.07% CPC (alcohol)</td>
<td>Antiplaque and antigingivitis effects in vivo. The subjects rinsed with 20 ml for 30 s twice daily during 2 weeks.</td>
<td>Gingival Index-GI, Plaque Index-PI and bleeding Index-BI</td>
<td>EO demonstrated significant reduction in GI, PI and BI compared to CPC.</td>
</tr>
<tr>
<td>Cortelli et al.</td>
<td>1. EO with zinc chloride and 0.02% sodium fluoride (alcohol) 2. 0.05% CPC + F</td>
<td>Antiplaque and antigingivitis effects in vivo. The subjects rinsed with 20 ml for 30 s twice daily during 6 months.</td>
<td>Gingival and Plaque Index</td>
<td>EO presented clinical superiority compared to CPC.</td>
</tr>
<tr>
<td>Costa et al.</td>
<td>1. 0.07% CPC (alcohol-free) 2. Saline-based solution (alcohol-free)</td>
<td>Treatment was done three times a day, for 6 months in vivo.</td>
<td>PG, BI and CFU counts (A. actinomyces, P. gingivais, P. intermedia/nigrescens, T. forsythia, P. micra, Capnocytophaga spp., E. corrodens, Eubacterium spp. and F. nucleatum)</td>
<td>Significant reduction of the clinical parameters was observed for the tested CPC solution. P. intermedia/CFU showed a clear reduction after 3 and 6 months of CPC treatment compared to control.</td>
</tr>
<tr>
<td>Ennibi et al.</td>
<td>1. 0.12% CHX (alcohol) 2. 0.1% CHX (alcohol-free) containing 0.1% formaldehyde</td>
<td>Antiplaque and antigingivitis effect for 7 days in vivo. The treatment was done twice daily</td>
<td>Plaque Indexes</td>
<td>CHX with alcohol showed better inhibition of plaque growth than CHX with formaldehyde.</td>
</tr>
<tr>
<td>Sánchez et al.</td>
<td>1. 0.12% CHX and 0.05% CPC (alcohol-free) 2. EO (alcohol)  3. 325 ppm of Amine fluoride and 125 ppm of stannous fluoride (alcohol-free) - AFSF</td>
<td>Bacteria related with periodontal disease in planktonic phase and multispecies Biofilm in vitro/treatment was done during 1 min (planktonic) and 2 min during 7 days (biofilm)</td>
<td>CFU counting</td>
<td>CHX/CPC and AFSF containing mouthrinses demonstrated superior antimicrobial activity compared to EO rinse.</td>
</tr>
<tr>
<td>Blanc et al.</td>
<td>1. 0.12% CHX plus 0.05% CPC; 2. 0.12% CHX 3. 0.02% CHX plus NaF</td>
<td></td>
<td>CFU counting for periodontal bacteria</td>
<td>In planktonic phase (S. oralis F. nucleatum, P. gingivais and A. actinomyces) no differences between the tested mouthrinses were found. For biofilm, CHX + CPC showed more inhibition of viable cells compared CHX and CHX + NaF.</td>
</tr>
<tr>
<td>Cortelli et al.</td>
<td>1. EO (alcohol) 2. 0.07% CPC (alcohol)</td>
<td>Antiplaque and antigingivitis effect in vivo/for 6 months/treatment was done twice daily</td>
<td>The MGI, PI and BI were quantified after 1, 3 and 6 months.</td>
<td>EO presented clinical superiority compared to CPC.</td>
</tr>
<tr>
<td>Elias-Boneta et al.</td>
<td>1. 0.075% CPC (alcohol-free and fluoride-free) 2. EO (alcohol)</td>
<td>The antiplaque and antigingivitis effect in vivo after 6 weeks/treatment was done twice daily</td>
<td>Pl and GI</td>
<td>Both mouthrinses proved a significant reduction in dental plaque and gingivitis.</td>
</tr>
<tr>
<td>Papaionnou et al.</td>
<td>1. 0.2% CHX (alcohol-free) 2. 0.2% CHX (alcohol)</td>
<td>The antiplaque and antigingivitis effect in vivo after 21 days/treatment was done during 1 min.</td>
<td>PI, GI and Discoloration Index</td>
<td>Both formulations presented comparable levels of action.</td>
</tr>
</tbody>
</table>

Abbreviations: CHX – chlorhexidine; EO – essential oil; CPC - cetylpyridinium chloride; GI – gingival index; PI – plaque index; BI – bleeding index; F – fluoride; CFU – colony forming units; ppm – parts per million; AFSF - amine fluoride/stannous fluoride; NaF – Sodium fluoride; MGI – Modified Gingival Index.
CONCLUSION

There are important differences in the antimicrobial performance among the commercial mouthrinses especially considering the bacteria specie (cariogenic vs. periodontopatogenic bacteria). More clinical studies are needed for better understanding the differences in their performance and side effects in short- and long-term studies.

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REFERENCES


