



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.

RESUMO

Esta revisão tem como objetivo discutir o potencial antimicrobiano de diferentes enxaguatórios bucais em relação ao controle da cárie dentária e doença periodontal. A pesquisa foi realizada usando PubMed e as seguintes palavras-chave: “agente antimicrobiano” ou “agente antiplaca”, “biofilme dental” e “cárie dentária” ou “doença periodontal” ou “gingivite”. Foram selecionados os estudos publicados em inglês, de 2011 a 2015, em revistas com fator de impacto maior que 0,8. Foram encontrados no total 22 artigos, 13 relacionados à cárie dentária e 9 relacionados à doença periodontal. Entre os 13 estudos envolvendo bactérias e/ou biofilme cariogênicos, 6 foram realizados *in vitro*, 3 *in situ* e 4 *in vivo*. Entre os 9 estudos envolvendo doença periodontal, 2 foram *in vitro* e 7 *in vivo*. Os principais agentes ativos testados foram: CHX-Clorexidina, CPC-cloreto de cetilpiridínio e OE-óleos essenciais (com álcool ou sem álcool). A CHX foi comparada ao OE em 6 estudos, mostrando superioridade em 3 estudos, similaridade em 1 estudo e inferioridade em 2 estudos. CPC mostrou menor efeito na redução da placa em comparação à CHX e ao OE. Ainda há controvérsias sobre o efeito do álcool, mas alguns estudos têm mostrado superioridade no caso de OE e CHX com álcool sobre biofilmes cariogênicos e periodontopatogênicos, respectivamente, quando comparados à versão sem álcool; para o CPC, não foi encontrada diferença. Mais estudos clínicos são necessários para melhor compreensão sobre mecanismo de ação e as diferenças de desempenho entre os agentes antiplaca.

PALAVRAS-CHAVE

Agentes antimicrobianos; Biofilme dental; Cárie dentária; Doenças orais; Periodontite.

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. actinomycetemcomitans*, *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

(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 (0.09% 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 ($4.8 \times 10^6 \pm 5.4 \times 10^6$ bacteria/cm²) than on enamel ($1.2 \times 10^6 \pm 1.5 \times 10^6$ bacteria/cm²). Chlorhexidine significantly reduced the amount of adherent bacteria (dentin: $2.8 \times 10^5 \pm 3.4 \times 10^5$ bacteria/cm²; enamel: $4.2 \times 10^5 \pm 8.7 \times 10^5$ bacteria/cm²). Rinses with the fluoride solution also significantly reduced bacterial adherence to dentin ($8.1 \times 10^5 \pm 1.5 \times 10^6$ bacteria/cm²). 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 pre-molar 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 (6.5×10^6 arbitrary units/mm² for control, 0.82×10^6 arbitrary units/mm² for CHX and 0.87×10^6 arbitrary units/mm² 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 I - Effect of commercial antimicrobial mouthrinses on dental caries or bacteria caries-related

Authors	Active component	Type of microorganism and model/ treatment	Response variable	Results
Guggenheim & Meier [18]	1. 0.1% CHX (alcohol) 2. 0.2% CHX (alcohol) 3. 0.12% CHX (alcohol-free) 4. 0.2% CHX (alcohol-free) 5. 0.05 CHX (alcohol) 6. EO (alcohol) 7. 0.15% CHX (alcohol-free)	Multispecies biofilm/ treatment of 1 min each 12h, during 64.5-h <i>in vitro</i>	Total microorganisms (<i>A. naeslundii</i> , <i>V. dispar</i> , <i>F. nucleatum</i> , <i>S. mutans</i> , <i>S. oralis</i> and <i>C. albicans</i>), <i>S. mutans</i> and <i>S. oralis</i> CFU numbers.	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 <i>S. mutans</i> in 7 log ¹⁰ steps compared to control (water). 0.1% CHX (alcohol), 0.2% CHX (alcohol), 0.15% CHX (alcohol-free) reduced the number of CFU of <i>S. oralis</i> in 3 log ¹⁰ steps compared to control (water).
Marchetti et al. [24]	1. EO (alcohol-free) 2. EO (alcohol)	Cariogenic biofilm/ treatment twice a day during 3 days <i>in vivo</i>	Plaque index-PI in whole mouth	EO (alcohol) showed better inhibitory effect compared EO (alcohol-free)
Sreenivasan et al. [22]	1. 0.05% CPC+ (alcohol) 2. 0.05% CPC- (alcohol-free) 3. 0.05% Sodium Fluoride (alcohol) 4. 0.12% CHX (alcohol)	(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	MIC and CFU of <i>S. gordonii</i> , <i>S. mutans</i> , <i>C. albicans</i> , <i>A. meyeri</i> , <i>A. viscosus</i> .	The cariogenic bacteria were inhibited by <6% CPC+ solution, <3% CPC- solution and <50% F solution. Both CPCs had similar effect and were better than F. For CFU, CPC mouthrinses (>90% killing) and CHX rinse (>98% killing) were better compared to F.
Ramalingam et al. [21]	1. 1% CPC and 10% Triton X-100-TRI (NE) 2. 0.12% CHX (alcohol-free)	<i>S. mutans</i> , <i>L. casei</i> , <i>A. viscosus</i> , <i>C. albicans</i> and mixed culture biofilm (72h).	Level of microorganisms adhesion on glass surface and MIC/ MBC values	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 <i>S. mutans</i> and <i>L. casei</i> 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.

Authors	Active component	Type of microorganism and model/ treatment	Response variable	Results
Oyanagi et al. [25]	1. 0.05% CHX (alcohol-free) 2. 0.2% benzethonium chloride 3. EO (alcohol) 4. 7% povidone iodine (PVP-I)	(A) <i>S. mutans</i> and <i>S. sobrinus</i> in planktonic phase (48-hour incubation). (B) Multispecies Biofilm (<i>S. mutans</i> , <i>S. sobrinus</i> and <i>S. gordonii</i>) / treatment (1x60s) for 7 days <i>in vitro</i>	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. EO and F 2. 0.12% CHX (alcohol-free)	Inhibition on <i>S. mutans</i> and <i>Lactobacillus</i> in saliva/ treatment twice a day for 16 days <i>in vivo</i>	CFU counting	CHX (alcohol-free) showed significant reduction in <i>S. mutans</i> and <i>Lactobacillus</i> , while the EO rinse did not.
Hanning et al. [29]	1. 0.2% CHX (alcohol-free) 2. 100 ppm AmF and 150 ppm NaF	Biofilm formation (8h) on enamel and dentin/ treatment for 1 min <i>in situ</i>	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. EO (alcohol) 2. EO (alcohol-free) 3. 0.1% CHX (alcohol-free)	Treatment for 4 days <i>in vivo</i> . Groups 1 and 3 rinsed 2x30s/day, while Group 2 rinsed 3x30s/day	CFUs counting for <i>S. mutans</i> on the teeth and tongue surfaces	All mouthrinses were similarly able to significantly reduce the number of <i>S. mutans</i> .
Babu and Garcia-Godoy [19]	1. 0.12% CHX plus 0.05% CPC (alcohol-free) 2. 0.12% CHX (alcohol-free) 3. 0.2% zinc chloride plus 1.5% hydrogen peroxide (alcohol-free)	<i>S. mutans</i> , <i>S. faecalis</i> , <i>S. gordonii</i> , <i>A. viscosus</i> in a mixed culture biofilm (48h)/ treatment during 1 min <i>In vitro</i>	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) 2. EO (alcohol) 3. CPC (alcohol-free) 4. Isopropyl methyl phenol (alcohol-free)	<i>S. mutans</i> biofilm/ treatment during 30s <i>in vitro</i>	Penetration velocities and antimicrobial effect by monitoring fluorescence loss of calcein 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 <i>S. mutans</i> .
Pandit et al. [28]	1. 0.025% CPC 2. 0.05% CPC 3. 0.075% CPC 4. 0.1% CPC 5. Negative control (water).	<i>S. mutans</i> biofilm/ treatment each 12h, during 98 h <i>in vitro</i>	Weight, viability and acidogenicity on Early (0h to 50h) and mature (48h to 98h) <i>S. mutans</i> 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)	Anti-plaque effect <i>in situ</i> / 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. 0.1% CHX (alcohol-free) 2. 0.2% NaF (alcohol-free)	Cariogenic biofilm <i>in situ</i> / 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.

Commercial Agents and Biofilm/Periodontal Disease

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*, *F. nucleatum*, *A. actinomycetemcomitans*, *P. gingivalis*) and on biofilm formed *in vitro*. The effect of the mouthrinses 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 $ZnCl_2$ 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 ($1.38 \times 10^8 \pm 8.54 \times 10^7$ CFU/mL), followed by AFSF ($1.42 \times 10^8 \pm 9.03 \times 10^7$ CFU/ml), EO ($1.67 \times 10^8 \pm 1.17 \times 10^8$ CFU/ml) and the negative control ($2.55 \times 10^8 \pm 1.63 \times 10^8$ 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.

Authors	Active component	Type of microorganism and model/treatment	Response variable	Results
Charles et al. [38]	1. EO (alcohol) 2. 0.07% CPC (alcohol)	Anti plaque and antigingivitis effects <i>in vivo</i> . The subjects rinsed with 20 ml for 30 s twice daily during 2 weeks	Gingival Index-GI, Plaque Index-PI and bleeding Index-BI	EO demonstrated significant reduction in GI, PI and BI compared to CPC.
Cortelli et al. [39]	1. EO with zinc chloride and 0.02% sodium fluoride (alcohol) 2. 0.05% CPC + F	Anti plaque and antigingivitis effects <i>in vivo</i> . The subjects rinsed with 20 ml for 30 s twice daily during 6 months	Gingival and Plaque Index	EO presented clinical superiority compared to CPC.
Costa et al. [12]	1. 0.07% CPC (alcohol-free) 2. Saline-based solution (alcohol-free)	Treatment was done three times a day, for 6 months <i>in vivo</i> .	PI, BI and CFU counts (<i>A. actinomycetemcomitans</i> , <i>P. gingivalis</i> , <i>P. intermedia/nigrescens</i> , <i>T. forsythia</i> , <i>P. micra</i> , <i>Capnocytophaga spp.</i> , <i>E. corrodens</i> , <i>Eubacterium spp.</i> and <i>F. nucleatum</i>)	Significant reduction of the clinical parameters was observed for the tested CPC solution. <i>P. intermedia</i> CFU showed a clear reduction after 3 and 6 months of CPC treatment compared to control.
Ennibi et al. [35]	1. 0.12% CHX (alcohol) 2. 0.1% CHX (alcohol-free) containing 0.1% formaldehyde	Anti plaque and antigingivitis effect for 7 days <i>in vivo</i> /The treatment was done twice daily	Plaque Indexes	CHX with alcohol showed better inhibition of plaque growth than CHX with formaldehyde.
Sánchez et al. [40]	1. 0.12% CHX and 0.05% CPC (alcohol-free) 2. EO (alcohol) 3. 125 ppm of Amine fluoride and 125 ppm of stannous fluoride (alcohol-free) - AFSF	<i>S. oralis</i> , <i>V. parvula</i> , <i>A. naeslundii</i> , <i>F. nucleatum</i> , <i>A. actinomycetemcomitans</i> <i>in a mixed biofilm</i> (72 hours)/ treatment was done for 1 min	CFU counting	CHX/CPC and AFSF containing mouthrinses demonstrated superior antimicrobial activity compared to EO rinse.
Blanc et al. [37]	1. 0.12% CHX plus 0.05% CPC; 2. 0.12% CHX 3. 0.12% CHX plus NaF	Bacteria related with periodontal disease in planktonic phase and multispecies Biofilm <i>in vitro</i> / treatment was done during 1 min (planktonic) and 2 min during 7 days (biofilm)	CFU counting for periodontal bacteria	In planktonic phase (<i>S. oralis</i> , <i>F. nucleatum</i> , <i>P. gingivalis</i> and <i>A. actinomycetemcomitans</i>) no differences between the tested mouthrinses were found. For biofilm, CHX+CPC showed more inhibition of viable cells compared CHX and CHX+NaF.
Cortelli et al. [11]	1. EO (alcohol) 2. 0.07% CPC (alcohol)	Anti plaque and antigingivitis effect <i>in vivo</i> for 6 months/treatment was done twice daily	The MGI, PI and BI were quantified after 1, 3 and 6 months.	EO presented clinical superiority compared to CPC.
Elias-Boneta et al., [41]	1. 0.075% CPC (alcohol-free and fluoride-free) 2. EO (alcohol)	The anti plaque and antigingivitis effect <i>in vivo</i> after 6 weeks/ treatment was done twice daily	PI and GI	Both mouthrinses proved a significant reduction in dental plaque and gingivitis.
Papaionnou et al. [36]	1. 0.2% CHX (alcohol-free) 2. 0.2% CHX (alcohol)	The anti plaque and antigingivitis effect <i>in vivo</i> after 21 days/ treatment was done daily during 1 min	PI, GI and Discoloration Index	Both formulations presented comparable levels of action.

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. periodontopathogenic 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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