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Safety and Efficacy of Antimicrobial Mouthrinses in Clinical Practice

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Efficacy Overview. The use of an antimicrobial mouthrinse is an important adjunct to toothbrushing and interdental cleaning. To varying degrees, chlorhexidine gluconate (CHG), cetylpyridinium chloride (CPC), and essential oils (EO) interrupt the integrity of the bacterial cell membrane, leading to lysis and death. CHG binds to salivary mucins, tooth structure, dental plaque, and oral soft tissues and is released slowly into the mouth, where it inhibits adsorption of bacteria onto teeth. CHG is active against a wide range of gram-positive and gram-negative microorganisms. CPC binds to teeth and plaque to a lesser degree than CHG and is generally less efficacious than CHG. CHG and EO penetrate plaque biofilm and produce changes in microbial cell surface morphology that alter coaggregation, recolonization, and, thus, survival. CHG, CPC, and EO are active against a wide variety of aerobic and anaerobic bacteria. An overview of the Food and Drug Administration and American Dental Association rigorous approval processes for efficacy and safety is provided.

Safety Overview. Long-term use of CHG or EO does not adversely affect the ecology of oral microbial flora, including microbial overgrowth, opportunistic infection, or development of microbial resistance. Long-term use of CHG, CPC, or EO does not contribute to soft tissue lesions or mucosal aberrations and has no serious adverse effect on salivary flow, taste, tooth deposits, or dental restoration. There is no evidence of a causal link between alcohol-containing mouthrinses and the risk of oral and pharyngeal cancer.

Keywords: Antimicrobial mouthrinse, efficacy, gingivitis, mechanism of action, safety

Introduction

Mechanical plaque removal through toothbrushing and flossing has been the universally accepted "gold standard" for maintaining oral health since the early 1960s. However, numerous studies have shown that most patients do not effectively clean interdentally to remove dental plaque daily.[1-3] By the early 1980s, chemotherapeutic agents were marketed as adjuncts to brushing and flossing; however, no definitive guidelines for the evaluation of their safety and efficacy were available. Both the American Dental Association (ADA) and the Food and Drug Administration (FDA) have established standards for assessing the safety and efficacy of over-the-counter (OTC) and prescription mouthrinses.

ADA Safety and Efficacy Guidelines for Mouthrinses

Since 1931, the ADA, through its voluntary Seal of Acceptance Program, has promoted the use of oral and dental products that are both safe and effective. Published guidelines developed by the ADAlist the acceptance criteria for each type of agent, product, or device. In order to obtain the Seal of Acceptance, a company must provide evidence establishing that a submitted agent, product, or device meets or exceeds the guidelines for that particular usage and is safe and effective. Additionally, the product must have been approved for marketing in the United States by the FDA. In 1985, the ADA recognized the potential benefits of some chemotherapeutic formulations, giving impetus to the development of guidelines for the evaluation of antiplaque and antigingivitis chemotherapeutic agents for inclusion in the Seal Program, which are still in use today.[4] In order to be awarded the Seal, an antiplaque and antigingivitis chemotherapeutic must[5]

- Be tested in populations of typical product users in a randomized, parallel-group, or crossover clinical trial in which the test product is compared with a negative control and, if appropriate, an active control
- Be supported by data from at least two 6-month studies conducted at independent sites, with assessment of gingivitis and qualitative and quantitative assessment of plaque performed at baseline, an intermediate point (usually 3 months), and 6 months
- Document a statistically significant reduction of supragingival plaque and gingivitis as compared with a negative control in each of the 2 studies and demonstrate a statistically significant reduction of gingivitis for the mouthrinse group of at least 15% for any one study and an average reduction of 20% in the 2 studies compared with the control group
- Establish product safety with respect to soft tissues, teeth, toxicology, and effects on the oral flora (eg, adverse shifts in microbial populations, the development of microbial resistance, and the emergence of opportunistic organisms)

Data from the studies are then presented to and reviewed by the ADA Council on Scientific Affairs. If the product meets the established standards, it is awarded the ADA Seal of Acceptance.[4,5]

For the professional and consumer, the ADASeal for antimicrobial mouthrinses indicates that

- Product data have successfully undergone an intensive, nonbiased safety and efficacy review
- Evidence supports the manufacturer's claim for effectiveness against supragingival dental plaque and gingivitis
- The product is safe when used as directed

FDA Regulation

The FDA regulates prescription drugs as well as any OTC products that make therapeutic claims, such as the reduction of gingivitis. The FDA has accepted key elements for gingivitis assessment used by the ADA Seal Program as appropriate for its review. However, in contrast to the ADA, which evaluates products, the FDA evaluates active ingredients while recognizing that the way in which an ingredient is formulated may affect its clinical activity. In 2003, the recommendations of the FDA's Dental Plaque Subcommittee of the Nonprescription Drugs Advisory Committee were published, and they included the conditions under which OTC products for the reduction or prevention of dental plaque and gingivitis would

be recognized as safe, effective, and not misbranded.[6,7] In addition to data supporting effectiveness, the following criteria are examined by the FDA[6]:

- Incidence and risk of adverse reactions and significant side effects when used according to adequate directions
- Margin of safety with normal use
- Potential for harm from abuse or misuse
- Potential for inducing adverse side effects (such as irritation, ulceration, inflammation, erosion, damage to teeth/restorations)
- Benefit-risk ratio

After assessing an OTC ingredient, the FDA assigns the ingredient to a category of I, II, or III [6,7]:

- Category I: The ingredient is both safe and effective and is not misbranded.
- Category II: The ingredient is not generally recognized as safe and effective or is misbranded.
- Category III: There are insufficient data to evaluate safety and/or effectiveness.

The FDA may also approve products, both prescription and OTC, through the New Drug Application (NDA) process. The NDA process is a more lengthy one that also requires documentation of both the safety and efficacy of the product.

Mouthrinses That Meet ADA and/or FDA Guidelines

Two antiseptic mouthrinses (and their generic equivalents) have been awarded the ADA Seal for chemotherapeutic control of supragingival plaque and gingivitis: 0.12% chlorhexidine gluconate (CHG) mouthrinse (Peridex®) and essential oils (EO) mouthrinse (Listerine®). Because of a recent change in the ADA Seal Program, Peridex® and its generic equivalents as prescription products no longer carry the ADASeal. However, no CPC formulation has yet to obtain the ADA Seal. (See also page 32 for more information on the ADA Seal Program.)

The FDA's Dental Plaque Subcommittee of the Nonprescription Drugs Advisory Committee has classified 2 OTC mouthrinse ingredients as both safe and effective and not misbranded (Category I): cetylpyridinium chloride (CPC; examples of products include Colgate Viadent® and Crest® Pro-Health™ Rinse) and EO.[6,7] CHG was reviewed and found to be safe and effective by the FDA by means of an NDA and is available in the United States only by prescription.

Although many commercial mouthrinse manufacturers claim antiplaque and antigingivitis properties, most lack the efficacy data required to earn the ADA Seal. Stannous fluoride has received Category I recommendation by the FDA's advisory committee, and triclosan has received NDA approval by the FDA. However, these agents are not found in mouthrinse formulations in the United States. This article discusses the safety and efficacy data of mouthrinses that have been approved by the FDA, recommended as Category I by the advisory committee, or awarded the ADA Seal.

KEY POINT: The ADA and FDA have rigorous approval processes

The ADA grants its Seal of Acceptance to mouthrinses that have documented safety and efficacy through at least 2 longitudinal, controlled clinical trials. The FDA evaluates OTC ingredients making therapeutic claims. It has adopted key elements for gingivitis assessment from the ADA Seal of Acceptance criteria and assigns categories (I, II, or III) based on level of safety and efficacy. For certain prescription mouthrinses, the FDA evaluates safety and efficacy via the New Drug Application (NDA) process.

Antimicrobial Mouthrinse Safety

Two essential criteria for any product are *safety* and *efficacy*. The most effective product would be useless if it were not safe; conversely, the safest product would be inconsequential if it did not work. Issues related to safety in mouthrinses include the following:

- Are there any adverse effects on the oral microbial flora?
- Are there any oral soft tissue aberrations?
- Does routine use adversely affect dental restorative materials?
- Are there any contraindications for the use of these products?

Each of these concerns merits careful consideration.

Do Mouthrinses Have Adverse Effects on Oral Microbiota?

Some dental professionals may fear that antiseptic mouthrinses pose a risk in killing or inhibiting normal flora with subsequent repopulation with opportunistic and/or more pathogenic or resistant organisms. The microbial shift would manifest as an overgrowth of opportunistic organisms, such as *Candida*. Fortunately, studies document no adverse effects on supragingival dental plaque microflora after 6 months of continued use with either CHG or EO.[8-12] Table I describes the findings of several studies of the impact of EO and CHG on normal oral flora. Evidence confirms that daily, long-term use (6 months or longer) of CHG or EO does not adversely affect oral microbial flora, including no microbial overgrowth, opportunistic infection, or development of microbial resistance.

Table I. Effect of CHG and EO on Normal Oral Flora

Mouthrinse	Study Description	Outcome	References
0.12% Chlorhexidine gluconate (CGH) and essential oils (EO)	Several studies of 6 months' duration or longer; dental plaque harvested at baseline, midpoint, and end. Minimum inhibitory concentration microbial samples taken	Routine use of CHG and EO did not cause adverse shifts in plaque ecology, emergence of opportunistic pathogens, or development of resistant microbial strains	8, 9,12
0.12% CHG and EO	*Candida* species (*C albicans, C dubliniensis, C krusei, C glabrata, C tropicalis*) grown in vitro and treated with 0.12% CHG or EO	Both agents effective against test fungal species at commercially available concentrations with comparable inhibition between CHG and EO	13
EO	Randomized, crossover study with 29 adults to determine whether regular antimicrobial rinse use had the potential for a selective increase of *Streptococcus mutans* or an overgrowth of fungal species. Participants rinsed with EO or placebo for 14 days	Reduction in *S mutans*: Recoverable *S mutans* counts from the participants' interproximal spaces reduced by 75.4% with EO compared with control. Total streptococci in interproximal plaque declined by 69.9%. EO activity 37.1% greater against *S mutans* than against other streptococci. No increase in risk of caries	14
EO	In vivo investigations in persons with denture stomatitis caused by an overgrowth of *C albicans* and other fungal species in maxillary prostheses	Rinsing with EO twice daily was as effective as nystatin oral suspension in reducing clinical palatal inflammation and candidiasis	15,16

Do Mouthrinses Cause Oral Mucosal or Other Soft Tissue Aberrations?

Concerns about potential adverse effects on oral mucosa and other soft tissue include the following:

- Does alcohol cause adverse effects such as an increased risk of oral and pharyngeal cancer (OPC)?
- Are the active ingredients found in CHG, CPC, and EO safe for long-term use on the oral mucosa?
- Do mouthrinses affect salivary flow?
- Are there adverse effects on taste or tooth deposits?

Several studies have addressed these issues and are discussed below.

Does alcohol cause adverse effects such as an increased risk of OPC? Many mouthrinses contain pharmaceutical-grade alcohol to solubilize active ingredients, make them biologically active, or dissolve flavoring agents. Typical alcohol levels in mouthrinses include the following:

- CHG: generally 12.6% alcohol
- CPC: 6% to 18% alcohol (traditional) and alcohol free, with high-bioavailability CPC, 0.07%17
- EO: 26.9% alcohol (original "gold" product) and 21.6% alcohol (flavored products)

Oral care professionals may be reluctant to recommend an alcohol-containing mouthrinse (ACM) because of perceived risk for developing OPC. It is well known that tobacco usage and excessive alcoholic beverage consumption cause a substantial portion of the OPC.[18-20] Since most mouthrinses contain alcohol, do ACMs increase cancer risk as well? A number of studies have examined a cause-effect relationship between ACMs and OPC with varying results.[19,21-27] A critical review of investigations that suggested a causeeffect relationship revealed a number of deficiencies and study design flaws that necessitate rethinking the ACM-cancer link[28,29]:

- Lack of a dose-response based on frequency and/or duration of mouthwash use
- Inconsistent findings among studies
- Lack of a scientific or biological basis to explain inconsistent findings between males and females
- Absence of correction for alcoholic beverage ingestion and tobacco use
- Inclusion of pharyngeal cancer, an improper classification as mouthrinses only contact the oral cavity
- Inclusion of other head and neck carcinomas, lymphomas, and sarcomas as oral cancer, an improper classification as mouthrinses only contact the oral cavity

KEY POINT: No link between ACMs and OPC

According to the FDA, National Cancer Institute, and ADA, there is no evidence of a causal relationship between ACMs and OPC.[6,28] Most mouthrinses accepted by the ADA as safe and effective contain alcohol. The ADA Seal documents a product's safety and efficacy, and the ADA recommends that patients continue to use antiseptic mouthrinses as advised by their dental hygienist and dentist.[28,34]

A widely referenced study by the National Cancer Institute erroneously concluded that OPC risks were elevated 60% among female and 40% among male users of mouthwash (with >25% alcohol).[27] This epidemiologic retrospective investigation consisted of interviews with 866 patients with OPC, diagnosed January 1984 through March 1985, and 1249 controls from the general population without OPC sampled from 4 areas of the United States. Reanalysis of this report by independent reviewers concluded that many patients in the OPC group (6.6% of men and 12.6% of women) had tumors of nonmucosal histology that could not have been contacted by an ACM. Reanalysis of the data showed no relationship between ACMs and OPC.[6,30,31] Additional investigators continue to report that there is no evidence that ACM use increases OPC risk.[28,32,33]

Data comparisons of topical alcohol exposure of the oral mucosa from ACMs and alcoholic beverage consumption may be invalid. Two or even 3 topical administrations of a 25% ACM, each lasting 30 seconds, seem unlikely to produce the same effect as long-term, habitual alcoholic beverage consumption. Pharmaceutical alcohol is not a carcinogen.[6,28] However, chemicals and additives found in alcoholic beverages can cause cancer; for example, urethane, a known carcinogen, is commonly found in alcoholic beverages.[6,19,28] Commercial mouthrinses contain pharmaceuticalgrade denatured alcohol (pure ethanol), which is free from contaminating carcinogens.

Taking the following precautions should limit any potential problems with ACMs:

- Advise patients to consult with their abuse sponsor (counselor) before using an ACM.
- EO is indicated for use in individuals over the age of 12 years. The effectiveness and safety of CHG have not been established in individuals under 18 years.35,36
- Use of an ACM in persons taking disulfiram (Antabuse®) and metronidazole (Flagyl®) is contraindicated, because in combination they may induce nausea, vomiting, and other unpleasant side effects.37,38

Do the active ingredients of CHG, CPC, and EO adversely affect the oral mucosa? Evidence supports that long-term use of CHG, CPC, or EO does not contribute to soft tissue lesions or mucosal aberrations. Longterm clinical trials (at least 6 months' duration) produced substantial evidence documenting the safety of the active ingredients of CHG, CPC, and EO mouthrinses on the oral mucosa and periodontium.[39-52] Complete oral soft tissue examinations were performed at each data collection period (baseline, 3 months, and 6 months) in these studies. Findings revealed no differences in the incidence or severity of adverse events between the CHG, CPC, or EO groups and control/placebo groups. With EO, users report an initial tingling/burning sensation that lessens rapidly with time and is considerably reduced by the addition of flavoring such as citrus.[29,42] A burning sensation and occasional mild desquamation have also been reported with CPC use.[53]

Do mouthrinses affect salivary flow? Xerostomia is a common side effect of many systemic diseases, radiation/chemotherapy, and numerous OTC and prescription medications. Amisconception is that the use of an ACM desiccates the oral mucosa, leading to xerostomia. However, studies have shown that rinsing with an EO mouthrinse does not induce mucosal drying or aberration.[54,55] Table II summarizes these study findings.

Table II. Effects of EO on Salivary Flow

Study Description	Outcome	References
Effect of EO versus placebo on the salivary flow rate and oral mucosa of 19 volunteers with documented xerostomia who used 3 rinses daily for 14 days followed by a cross-over after a 7-day washout period. Pre- and postrinse salivary flow rates were measured and oral soft tissues examined for evidence of irritation and inflammation	Under exaggerated conditions (3 rinses/day instead of the recommended 2), no lesions attributable to EO observed in the majority of patients. No statistically significant differences detected between pre- and postrinse salivary flow rates for either the EO or control group	54
Effect on salivary flow or symptoms of dry mouth of an EO mouthrinse and a non–alcohol-containing mouthrinse	No significant effect on salivary flow or dry mouth between the 2 groups	55

Are there adverse effects on taste and tooth deposits? Some patients may experience a bitter taste with EO use.[56] Taste alteration, as well as increased supragingival calculus formation and brown staining of the teeth and tongue, is associated with use of CHG and CPC.[42,46,56-60] CHG stains teeth, esthetic restorations, and implant abutments, and this staining can be problematic in a society that desires cosmetic dentistry and whiter and brighter teeth.[36,56]

Does Routine Use of Mouthrinses Adversely Affect Dental Restorative Materials?

A number of studies have addressed the concern raised about the effect of antimicrobial mouthrinses on dental materials. Other than the potential for staining with CHG and CPC, there are no documented adverse effects on dental materials. Table III summarizes the findings of these studies.

Table III. Effects of Antimicrobial Mouthrinses on Dental Materials

Mouthrinse	Study Description	Outcome	References
Seven mouthrinses (5 alcohol-containing mouthrinses [ACMs], 1 alcohol free, and 1 plain water)	In vitro study of resin specimens placed in 1 of 7 mouthrinses and vibrated for 30 seconds or 1 minute twice daily (to simulate actual use exposure times) for 180 days	No statistical difference among the tested solutions. ACMs caused no increased reduction in composite resin hardness	61
Essential oils (EO)	In vitro study measured effect of EO on resin bond strength on human teeth embedded in dental stone. Tooth surfaces etched and rinsed for 30 seconds with distilled water or various EO dilutions. Each tooth was then dried, a film of adhesive resin applied followed by composite resin, and shear bond strength (SBS) recorded	No differences in SBS found between the EO and control groups at all dilutions. EO had no effect on resin bond strength	62
EO	Direct effect of EO use on dental materials. Specimens of amalgam, glass ionomer, and composite subjected to EO or distilled water for a continuous 10-day period. For each material, compressive strength and water fluid absorption were compared; surface porosity was evaluated with scanning electron micrographs (SEM). Also, 10 subjects wore appliances with implanted study materials and rinsed twice daily for 30 seconds with EO or placebo. After 10 days, dental materials examined by SEM	No significant differences between the EO and control groups detected in vitro or in vivo. EO use had no adverse effect on restorative materials tested	63

KEY POINT: CHG, CPC, and EO cause no serious adverse effects in a generally healthy population when used according to directions

This includes effects on salivary flow, taste, tooth deposits, and dental restorations. Some users may experience minor taste alteration, staining, and supragingival calculus formation with some CHG and CPC formulations.

Efficacy of Mouthrinses

How Antimicrobial Mouthrinses Work

Antiseptics are chemical agents used to eliminate oral microorganisms in a variety of ways:

- By producing cell death
- By inhibiting microbial reproduction
- By inhibiting cellular metabolism

Most antiseptic agents are bactericidal, although some are bacteriostatic. The effectiveness of these agents varies widely and is dependent upon product formulation, concentration of the active agent, dose, substantivity, compliance, and interactions with other chemicals present in the oral cavity at the time of use. Different antimicrobial mouthrinses have demonstrated efficacy against bacteria, fungi, viruses, and spores. Some products produce a wide spectrum of activity, while others are effective against selected microorganisms only.[56] Notably, most studies, including longitudinal trials, testing the efficacy of CHG used the commercial product Peridex®, and Listerine® was the EO commercial product used

for all studies cited in this paper. CPC commercial preparations used in research studies vary by product concentration and brand.

Mechanism of action of CHG. CHG (0.12%) is a bactericidal bisbiguanide antiseptic, with demonstrated efficacy against the following organisms:

- A wide range of gram-positive and gram-negative organisms64
- Aerobes and anaerobes, many of which are associated with plaque and gingivitis, including Fusobacterium and Prevotella intermedia65
- Herpes simplex virus 1 and 2, human immunodeficiency virus 1, cytomegalovirus, influenza A, parainfluenza, and hepatitis B.12,66,67 CHG is not approved for the prevention and treatment of viral infections
- Seven species of Candida and other yeasts13,68,69 (often used alone or in combination with other antifungal medications to reduce opportunistic infections in at-risk populations, such as those undergoing treatment for leukemia or bone marrow transplantation70,71)

Exposure to CHG causes rupturing of the bacterial cell membrane, which allows for leakage of the cytoplasmic contents, resulting in cell death.[72,73] CHG binds to salivary mucins, reducing pellicle formation and inhibiting colonization of plaque bacteria.[64,74] It also binds to bacteria, which inhibits their adsorption onto the teeth.[64] CHG has been shown to penetrate the dental plaque biofilm, which enables CHG to access and kill pathogens embedded within the biofilm.[72]

CHG binds tightly to tooth structure, dental plaque, and oral soft tissues. It is released slowly into the mouth, which allows antimicrobial effects to be sustained for up to 12 hours, thus its high degree of substantivity.[64,75] A 30-minute interval is optimal between toothbrushing and rinsing with CHG to avoid an interaction between the positively charged detergents found in dentifrices (eg, sodium lauryl sulfate) and the cationic CHG rinse. This interaction, and possible inactivation of CHG, can also occur with the anionic fluoride ion found in stannous fluoride and in some toothpastes and mouthrinses.[73,76]

Mechanism of action of CPC. CPC, a quaternary ammonium compound, demonstrates bactericidal activity. Its mechanism of action is similar to CHG in that it ruptures the bacterial cell wall membrane, resulting in leakage of the intracellular contents and eventual cell death. CPC is also thought to alter bacterial metabolism and inhibit cell growth.[73, 77]

CPC binds to tooth structure and dental plaque biofilm; however, the degree of binding is not as strong as with CHG. Further, CPC is rapidly released from binding sites, which explains why it is generally less efficacious than CHG.[73] Like CHG, this cationic rinse may adversely interact with other charged ions found in dentifrices and mouthrinses, possibly limiting its biological activity.

Published data regarding the efficacy of CPC-containing mouthrinses are limited. In the United States, CPC is available in 2 concentrations: 0.05% found in cosmetic mouthrinses (Cepacol® and Scope®) and 0.07% found in therapeutic mouthrinses (BreathRx® and Crest® Pro- Health™ Rinse). It has been suggested that the unique vehicle found in Crest® Pro-Health™ Rinse is purported to increase the product's oral bioavailability when compared with other CPC-containing mouthrinses.[78]

In vitro studies have documented that CPC can be effective against the following organisms:

- Actinomyces viscosus, Porphyromonas gingivalis, Campylobacter rectus, Streptococcus sanguis, Eikenella corrodens, Salmonella typhimurium, Fusobacterium nucleatum, Haemophilus ctinomycetemcomitans, Lactobacillus casei, and P intermedia78
- Several species of Candida68,69,79-81

CPC, like CHG, has been suggested as a possible agent for the prevention and treatment of fungal infections. However, CPC mouthrinses may adversely affect systemic azole drug treatment of oropharyngeal candidiasis in immunocompromised persons. This negative outcome may be attributed to either a cross-resistance to the azole drugs against CPC-resistant

organisms or drug antagonism between CPC and azole antifungal medications when they are used in combination.[82] Two of 5 fluconazole-resistant C albicans strains have also exhibited reduced susceptibility to CPC.[82]

Mechanism of action of EO. EO antiseptic mouthrinse is a bactericidal combination of phenolic essential oils, including eucalyptol, menthol, methyl salicylate, and thymol. Phenolic compounds exert their antimicrobial effects by the following mechanisms[77, 83-87]:

- Cause protein denaturation
- Alter the cell membrane, resulting in leakage of the intracellular contents and eventual cell death
- Alter bacterial enzyme activity
- Exhibit anti-inflammatory properties by inhibiting prostaglandin synthetase, an enzyme involved in the formation of prostaglandins, which are primary inflammatory mediators. Note that the anti-inflammatory effect of phenolic compounds occurs at concentrations lower than those needed for antibacterial activity
- Cause perforation of the cell membrane and rapid efflux of intracellular contents (especially thymol)
- Alter neutrophil function by suppressing the formation of and scavenging existing free radicals generated in neutrophils and by altering neutrophil chemotaxis (especially thymol)

A 30-second exposure time to EO produces morphologic cell surface alterations in a variety of oral pathogens that suggest the loss of cell membrane integrity.[88] Cell surface changes may also alter bacterial coaggregation and recolonization that could potentially affect the growth and metabolism of these organisms. Microscopic evidence of cell surface roughening was obtained for the following microorganisms:

- C albicans
- F nucleatum
- A viscosus
- A viscosus
- S sanguis

Cell surface changes that result from a short exposure time to EO may adversely affect bacterial and fungal survival.[88] Exposure to levels of EO sublethal to microorganisms also reduces bacterial coaggregation with gram-positive pioneer species, an essential step in plaque maturation and the development of the complex pathogenic flora found in gingival disease. Decreased bacterial coaggregation reduces the rate of plaque maturation, which in turn may result in a decreased plaque mass, as is observed clinically with EO use.[89] EO also has been shown to extract endotoxins from gramnegative bacteria.[90] Endotoxins play an important role in pathogenesis; thus, reduction in endotoxin level should manifest as a decrease in gingival inflammation.

Unlike other OTC mouthrinses, EO has been shown to penetrate the dental plaque biofilm and is active against bacteria embedded within the biofilm.[72,91-93] EO kills a wide variety of aerobic and anaerobic bacteria\ associated with plaque biofilm and gingivitis, including the following[94]

- A actinomycetemcomitans
- A viscosus
- S mutans
- S sanguis
- Bacteroides species

Efficacy against gram-positive and gram-negative organisms occurs even at concentrations that are less than full strength.[94,95] A single 30-second rinse reaches and exerts an antibacterial effect interproximally, an important consideration given that gingival disease starts between the teeth and that individuals often cannot access interproximal areas with mechanical plaque removal techniques such as toothbrushing and flossing. Total recovered bacteria from proximal tooth surfaces was 43.8% lower following a single 30-second rinse of EO compared with a control (P=.001).[96] Rinsing twice daily with EO as an adjunct to brushing for 11 days reduced total recoverable streptococci in interproximal plaque by 69.9% (P<.001), with EO producing a 37.1% greater activity against *S mutans* than other streptococci. A significant reduction of 75.4% in total recoverable *S mutans* count was observed (P<.001).14 Studies also have demonstrated significant suppression of the oral flora for several hours after rinsing, documenting that the antimicrobial activity of EO extends beyond the rinsing period.[97-99]

In vitro studies have shown that EO is also active against viruses, including herpes simplex virus 1 and 2, hepatitis B, human immunodeficiency virus 1, and influenza Avirus, as well as against 7 species of *Candida*.[13,67,100] Like CHG, EO is not approved for the prevention and treatment of viral infections.

Unlike CHG and CPC, EO has a neutral electrical charge and does not interact negatively with other charged ions found in dentifrices and mouthrinses.[73] Moreover, its action is not inhibited by proteins in blood serum that inactivate many antimicrobial agents, including CHG.[94,95]

Efficacy of Mouthrinses on Plaque Biofilm and Gingivitis

The primary indication for antimicrobial mouthrinse use is the reduction of supragingival plaque biofilm and gingivitis in patients. A recent meta-analysis of 6-month clinical trials to evaluate the efficacy of a variety of antiplaque and antigingivitis products revealed that the largest body of studies supported the efficacy of EO.[101]A smaller body of studies supported the antiplaque and antigingivitis efficacy of 0.12% CHG. Results regarding the efficacy of CPC varied and were dependent upon product formulation.[101] Efficacy studies of CHG, CPC, and EO are summarized in Tables IV, V, and VI, respectively.

Table IV. Effects of CHG on Supragingival Plaque and Gingivitis

Investigator	Trial Length (months)	No. of Subjects	Concentration of CHG (%)	Plaque Decrease (%)	Gingivitis Decrease (%)
Löe et al, 1976[49]	24	120	0.20	45	27
Lang et al, 1982[50]	6	158	0.10 0.20	16.2 19.4	66.6 80.4
Segreto et al, 1986[102]	3	600	0.12 0.20	36 28	37 28
Grossman et al, 1986[48]	6	430	0.12	61	39
Grossman et al, 1989[47]	6	481	0.12	49	31
Brightman et al, 1991[103]	3	34	0.12	64.9	60.0
Overholser et al, 1990[42]	6	124	0.12	50.3	30.5
Eaton et al, 1997[104]	3	121	0.12	28	25
Charles et al, 2004[46]	6	108	0.12	21.6	18.2

Table V. Effects of CPC on Supragingival Plaque and Gingivitis

Investigator	Trial Length (months)	No. of Subjects	Concentration of CHG (%)	Plaque Decrease (%)	Gingivitis Decrease (%)
Allen et al, 1998[105]	6	111	0.05	28.2	24.0
Mankodi et al, 2005[51]	6	139	0.07	15.8	15.4
Stookey et al, 2005[52]*	6	366	0.075	17	23
			0.10	19	20

* The mouthrinse formulations in this study were experimental.

Table VI. Effects of EO (Listerine®) on Supragingival Plaque and Gingivitis

Investigator	Trial Length (months)	No. of Subjects	Rinsing Supervision	Plaque Decrease (%)	Gingivitis Decrease (%)
Lamster et al, 1983[40]	6	145	Supervised	22	28
Gordon et al, 1985[39]	9	85	Supervised	19.5	23.9
DePaola et al, 1989[41]	6	107	Supervised	34	34
Overholser et al, 1990[42]	6	124	Supervised	36.1	35.9
Charles et al, 2001[43]	6	316	Unsupervised	56.1	22.9
Bauroth et al, 2003[44]	6	326	Unsupervised	21	12
Sharma et al, 2004[45]	6	237	Unsupervised	51.9	21.0
Charles et al, 2004[46]	6	108	Unsupervised	18.8	14.0

The following observations can be made from these study results:

- CHG generally reduces more plaque than either CPC or EO, a predictable outcome given its greater substantivity; the longer an antimicrobial agent stays in contact with plaque bacteria, the greater its effect.
- CHG and EO are comparable in reducing gingivitis.39-41,43-45,48-50,102-104
- In head-to-head comparison studies that evaluated both CHG and EO in the same participants, antiplaque effects were greater for CHG, but antigingivitis effects were similar for both agents.42,46,47
- Both CHG and EO demonstrate greater reductions in supragingival plaque and gingivitis as compared with CPC (see Tables IV, VI).

Perhaps one EO study best summarizes the effectiveness of mouthrinses as an aid to reducing supragingival plaque and controlling gingivitis. In a large, randomized, controlled clinical trial involving 237 participants, those who added twice-daily rinsing with EO to their homecare routine of regular brushing and flossing demonstrated a 51.9% greater reduction in plaque and a 21.0% greater reduction in gingivitis, as compared with those who brushed and flossed only.[45] This study demonstrates the benefit of adding an EO mouthrinse to regular mechanical plaque removal and shows that mouthrinses are able to reach bacteria in areas that are difficult to access and where mechanical methods often leave residual plaque behind.

Approved Mouthrinses Are Efficacious Throughout the Entire Mouth

Using an antiseptic mouthrinse produces an antimicrobial effect throughout the entire mouth, including areas easily missed during toothbrushing and interdental cleaning. Studies have demonstrated that antiseptics kill bacteria in saliva and on the soft tissues of the mouth, including the tongue and oral mucosa, which are reservoirs of pathogenic bacteria that are able to transfer and colonize onto the teeth.[98,105-108] These collective research findings, with consideration given to the respective

adverse events profiles of antiseptic agents, reinforce the value of using CHG, CPC, and EO in addition to mechanical plaque control for longterm maintenance of gingival health.

Conclusion

Antimicrobial mouthrinses that are approved by the FDA and carry the ADA Seal of Acceptance are safe and effective for the reduction of supragingival plaque and gingivitis. Products that have not been evaluated in longterm clinical trials have no scientific evidence documenting safety or efficacy and should be used with caution. Antimicrobial mouthrinses with established safety and efficacy are an important and effective addition to mechanical plaque control methods to establish a healthy mouth. Most patients will benefit by adding an ADA-Accepted antimicrobial mouthrinse to their self-care daily regimen of brushing and interdental cleaning.

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