

The effect of mouthrinses against oral microorganisms

Ação dos enxagatórios bucais contra microrganismos bucais

José Roberto Cortelli^(a)
Raul Emilio de La Sotta Thénoux^(b)

^(a)Vice-Provost of Research, Department of Dentistry, Periodontics Research Division, University of Taubaté, Taubaté, SP, Brazil.

^(b)Dean of Graduate studies, Universidad Central de Chile, Santiago, Chile.

Abstract: This paper reviews the benefits of the use of antimicrobial mouthrinses for controlling dental biofilm. It is currently known that the human oral cavity is inhabited by approximately 600 to 700 different species of microorganisms, including bacteria, fungi and viruses, organized in biofilms. Biofilm accumulation represents the principal etiologic agent of oral diseases including caries and periodontal diseases. For that reason, prevention of biofilm accumulation has been shown to be associated with the control of diseases. Patient motivation and oral hygiene instruction are claimed to be a major factor influencing the degree of biofilm control. Therefore, mechanical home-care methods, including toothbrushing and flossing, represent the best way for patients to remove biofilm. For many patients, however, the elimination of all biofilm present in the oral cavity through home-care methods could be tedious and time-consuming. Additionally, some local conditions, including malpositioned teeth, presence of bridge-work or orthodontic appliances, among others, may render the mechanical control of biofilm especially difficult. Chemotherapeutic agents, including mouthrinses, could have a key role as adjuncts to daily home care, preventing and controlling supragingival plaque, gingivitis and oral malodor. Indeed, according to the ADA, the active ingredients of mouthwashes must demonstrate a therapeutic effect *in vivo* and *in vitro* to be classified as drugs. Several studies included in the present review have demonstrated the effectiveness of mouthrinses containing active ingredients such as chlorhexidine and essential oils in preventing and controlling both supragingival plaque and gingivitis, especially when used adjunctively to regular toothbrushing and flossing procedures.

Descriptors: Dental plaque; Oral hygiene; Anti-infective agents, local; Chlorhexidine.

Resumo: O propósito desta revisão foi mostrar os benefícios decorrentes do uso de enxagatórios bucais no controle do biofilme dental. Atualmente sabe-se que a cavidade bucal dos humanos é habitada por aproximadamente de 600 a 700 espécies microbianas incluindo bactérias, fungos e vírus. Este aglomerado microbiano, atualmente definido como biofilme dental, é o principal agente etiológico das patologias bucais, incluindo cárie e doença periodontal. Logo, cuidados em relação ao controle destas doenças passam necessariamente pela eliminação ou redução do biofilme dental, incluindo métodos mecânicos adequados de higiene bucal e motivação do paciente. Todavia, a escovação dental e o uso de fio dental podem ser uma tarefa tediosa e consumir tempo exagerado para a rotina de muitos pacientes. Além disso, o controle mecânico do biofilme dental pode ser dificultado por fatores locais como uso de próteses e aparelhos ortodônticos, mau posicionamento dental, entre outros. Assim, o uso de enxagatórios bucais, associado ao controle mecânico, pode ser incorporado rotineiramente aos cuidados de higiene bucal controlando terapêutica e preventivamente o biofilme supragengival, a gengivite e a halitose. De acordo com a ADA, agentes ativos vinculados aos enxagatórios bucais devem demonstrar efeitos terapêuticos *in vivo* e *in vitro* para que estes sejam classificados como droga. Diferentes estudos incluídos nesta revisão demonstraram que a clorexidina e os óleos essenciais podem, em associação ao hábito regular de escovar os dentes e usar corretamente o fio dental, prevenir e controlar tanto o biofilme supragengival quanto as gengivites.

Descritores: Placa dentária; Higiene bucal; Antiinfecciosos locais; Clorexidina.

Corresponding author

José Roberto Cortelli
Pró-Reitoria de Graduação, Departamento de Odontologia, Universidade de Taubaté
Rua dos Operários, 9 - Centro
Taubaté - SP - Brazil, CEP: 12020-240
E-mail: cortelli@iconet.com.br

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Introduction

Since 400 years ago, when Anthony van Leeuwenhoek began using microscopes to study the human body, samples of dental plaque have been studied by several researches around the world. Nowadays, many studies confirm that the mouth is a complex ecosystem, which contains many microbial inhabitants including bacteria, viruses and fungi. These microorganisms grow in thin layers on the mouth's surfaces as teeth, tongue and mucosa membrane. Today, these layers are called biofilm.

Biofilms are spatially-organized communities of bacteria attached to a surface and enclosed in a matrix of extra-cellular material derived from both the component species and the environment.¹¹ Biofilm exists to allow microorganisms to stick to surfaces and to multiply, representing the preferred method of growth for many bacterial species³⁸ and providing a number of advantages to colonizing species. Protection from both competing microorganisms and environmental factors, such as host defense mechanisms, is the main advantage to colonizing species, including protection from potentially toxic substances such as chemicals and antibiotics.⁴³

Dental biofilm

Biofilms are composed of microcolonies of bacterial cells non-randomly distributed in a shaped matrix or glycocalyx composed of water and aqueous solutes (exopolysaccharides, proteins, salts and cell material). It has been shown that there are specific associations between bacteria in dental biofilm. Inside biofilms one can observe the presence of water channels between microcolonies permitting the passage of nutrients and other agents throughout the biofilm, acting as a primitive circulatory system. Nutrients make contact with the attached microcolonies by diffusion from the water channel to the microcolony rather than from matrix.⁴³

Mature dental plaque is inhabited by approximately 600 to 700 species of bacteria at 10^8 – 10^9 bacteria per ml of saliva or mg of dental plaque.^{21,40} It is plausible that the presence of bacteria represents the principal etiologic agent for several oral diseases including caries and periodontal diseases.

Caries is a disease caused by species such as *Streptococcus mutans* and *Streptococcus sobrinus*.²⁷ In addition, bacterial species such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum* have frequently been isolated from periodontal lesions and have been shown to be related to the onset and progression of periodontal disease.^{42,44,46} On the other hand, microbial studies and clinical examinations have revealed that certain subgingival microorganisms are essential pathogenic factors in various types of human periodontal disease, so bacterial colonization may play an important role in the pathogenesis of periodontal disease.⁴⁹

Prevention of biofilm accumulation has been shown to be associated with the control of diseases.³ Mechanical home-care methods have long been regarded as the best way for patients to remove biofilm. On the other hand, if oral hygiene is neglected, plaque growth reaches a maximum extent within 3-4 days, and biofilm is detected on almost all tooth surfaces.²⁵ Patient motivation and oral hygiene instruction are claimed to be a major factor influencing the degree of biofilm control.^{39,47} Additionally, elderly patients, individuals presenting physical limitations, individuals using orthodontic appliances, bridge-work or presenting malpositioned teeth may find brushing and interdental cleaning especially difficult.¹⁰ Sometimes flossing also is widely time consuming and difficult for some patients. Since interdental hygiene is technically demanding, it is performed on a daily basis by only about 10% of the population.^{4,39}

For all these different reasons, the regular use of antimicrobial mouthrinses may play a key role as adjuncts to brushing and flossing for preventing caries and gingivitis.⁸

In order for an active ingredient to be classified as a drug, the ingredient must demonstrate a therapeutic affect. The American Dental Association (ADA) acceptance program requires that over-the-counter toothpastes or mouthrinses with plaque control claims must show concomitant gingivitis and plaque reduction. The ADA confirms that if a product can only demonstrate a significant plaque reduction without a concomitant significant reduction in gingivitis, it will not be eligible for the seal of acceptance.²

Several studies have demonstrated the effectiveness of mouthrinses containing active ingredients such as chlorhexidine and essential oils in preventing and controlling both supragingival plaque and gingivitis, specially when used adjunctively to toothbrushing and flossing procedures.^{14,26,32} Both chlorhexidine's and essential oils' action time are equal, acting for 12 hours against biofilm.¹⁶ Pan *et al.*³³ (2000) showed that bacterial phenotypes may change when the organisms go from a free floating state to a state where they are part of a biofilm. This change can result in altered susceptibilities to antimicrobial agents. Consequently, the efficacy of any antimicrobial mouthrinse depends not just on its microbicidal properties, that are often demonstrated *in vitro*, but also on its ability to penetrate the biofilm *in vivo*,³¹ therefore, we can confirm that this ability is what makes the difference. On the other hand, mouthrinses may not have efficacy in subgingival plaque and penetrate the subgingival area only minimally.⁵ Indeed, antimicrobial mouthrinses can not be expected to provide a therapeutic benefit by themselves in the treatment of periodontitis.³⁶

Chlorhexidine attacks the bacterial cell membrane, causing leakage and/or precipitation of the cellular contents.¹⁵ Chlorhexidine may be considered the gold standard of chemotherapeutic agent for plaque and gingivitis control.¹ The antibacterial activity of chlorhexidine is related to the cationic chlorhexidine molecule, which is rapidly attracted by the negatively charged bacterial cell surface. After adsorption, the integrity of the bacterial cell membrane is altered, which results in a reversible leakage of bacterial low molecular-weight components at low dosage¹² or more severe membrane damage at higher doses.³⁸ On the other hand, the adverse effects associated with chlorhexidine-containing mouthrinses have been related to extrinsic staining of the teeth and tongue, increased calculus formation and alteration of the taste sensation.⁴⁸ Therefore, long-term use should be indicated and supervised by the dental professional.

Cetylpyridinium chloride is a quaternary ammonium classified as a cationic surface active agent. Cetylpyridinium chloride acts primarily by penetrating

the cell membrane, which causes leakage of components in the cell, disruption of bacterial metabolism, inhibition of cell growth and cell death.⁶ However, because the positively charged hydrophilic region of cetylpyridinium chloride is critical to its antimicrobial activity, mouthrinse formulations should not contain ingredients that diminish or compete with the activity of this cationic group.⁴⁸

Essential oils mouthrinses act killing microorganisms by disrupting their cell walls and by inhibiting their enzyme activity.^{15,24} Antimicrobial mouthrinses containing essential oils have a combination of thymol (0.064 percent), menthol (0.042 percent), eucalyptol (0.092 percent) and methyl salicylate (0.060 percent), as active ingredients. Listerine (Johnson & Johnson Consumer & Personal Products Worldwide, Morris Plains, New Jersey, USA) was tested for efficacy against oral bacteria as early as 1884, by the legendary W. D. Miller. In his paper "Microorganisms of the human mouth", Miller stated that "Listerine has proved to be a very useful and active antiseptic".²⁸ In 1929, an independent assessment of this essential oils mouthrinse showed it to have significant bactericidal activity against a variety of microorganisms and concluded it to be safe and effective.

The combination of essential oils has shown to be safe and effective as an over-the-counter antiplaque and antigingivitis agent. In an *in vitro* study, Ross *et al.*⁴¹ (1989) showed that essential oils killed a wide range of microorganisms within 30 seconds in the presence of serum. Jenkins *et al.*¹⁹ (1994) showed that in an *in vivo* single rinse model, the use of an essential oils mouthrinse can significantly reduce the levels of recoverable salivary bacteria compared to a negative control rinse for periods of up to 5 h. Although some experienced an initial burning sensation and bitter taste, accommodation usually occurred in a few days and occasional tooth staining has been noted, but most clinical studies did not report this condition.⁴⁸ Clinical studies have shown that essential oils retarded the formation of supragingival plaque and decreased the degree and severity of gingivitis. Additionally, the antimicrobial activity of the essential oil mouthrinses has been shown in saliva,¹³ in the gingival crevice, and on the dor-

sal surface of the tongue.^{34,35} Plaque reduction has ranged from 20% to 34%, and gingivitis reduction, from 28% to 34% when essential oils were used twice daily following tooth brushing.^{17,18}

About 80 to 90% of the oral malodor originates locally in the mouth, and the bacterial overgrowth is the major etiological factor. In many cases, bacterial overgrowth is associated to poor oral hygiene, gingivitis and periodontitis, coating on the tongue, cavities and throat infections.²⁹ Additionally, oral malodor affects from 40 to 50% of the population in different levels.³⁰ *In vitro*, oral bacteria, as well as oral specimens such as saliva, plaque and tongue coatings, can produce the volatile sulfur compounds including hydrogen sulfide, methyl mercaptan, dimethyl sulfide, as well as compounds such as indoles and skatoles²² that contribute to the complex mixture of odorous molecules found in the exhaled air. *In vivo*, factors that support the growth of these bacteria will predispose a person to malodor. Examples include accumulation of food within pockets around the teeth, among the bumps at the back of the tongue, or in small pockets in the tonsils.^{23,37} Some studies have recognized that a diminished saliva flow also represents an important factor to malodor. A reduced saliva flow increases the concentration of bacteria in the mouth and worsens bad breath.⁴⁵

The tongue represents a special place for the colonization of microorganisms, including dental and periodontal pathogens. Indeed, the tongue is colonized immediately after birth and anaerobic species can be detected prior to the eruption of teeth. Their

numbers and the presence of other anaerobes increase at the time of the primary eruption of teeth.

To eliminate oral malodor, home oral hygiene procedures are recommended to reduce accumulations of debris and the presence of bacteria. These procedures include tooth brushing and flossing, brushing the tongue or using a tongue scraper. Mouthrinses act against the malodor caused by oral bacteria when used as a complement to mechanical means, since by controlling bacteria it is possible to control malodor. The effects of mouthrinses could be due to thymol and eucalyptol, the volatile oils that have proven activity against bacteria. Kato *et al.*²⁰ (1990) showed that bacterial counts plummet in as little as 30 seconds following a mouthrinse with thymol and eucalyptol. However, if the etiology of the oral malodor is related to periodontal disease, regular dental care is recommended to prevent or treat these pathologies.^{7,9}

Conclusions

As biofilm accumulation represents the principal etiologic agent for oral diseases, including caries and periodontal diseases, prevention of biofilm accumulation, patient motivation and oral hygiene instruction are claimed to be the major factors influencing the degree of caries and periodontal diseases control. This review shows that mouthrinses containing active ingredients such as chlorhexidine and essential oils represent an important tool to prevent and control both supragingival plaque and gingivitis, especially when used adjunctively to toothbrushing and flossing procedures.

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